

LASER INDUCED FLUORESCENCE IN ALGAE: A NEW TECHNIQUE FOR REMOTE DETECTION

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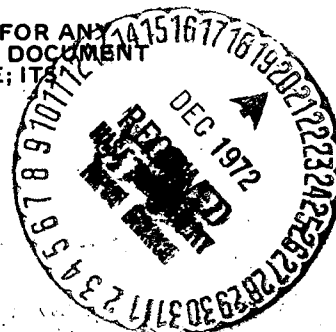
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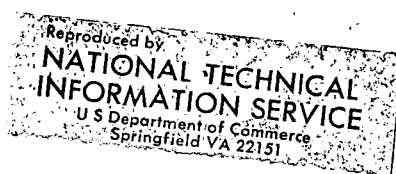
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WALLOPS STATION
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FINAL REPORT
October, 1972

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FOR REMOTE DETECTION

by

E. J. Friedman
G. D. Hickman

Sponsored by

National Aeronautics and Space Administration
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III

SUMMARY

Measurements of the absorption and fluorescence spectra were obtained for four various types of marine and fresh water algae using a pulsed N_2/Ne dye laser as the source of excitation. The absorption maxima for the algae ranged from 420 to 675 nm, while their fluorescent spectra ranged from 580 to 685 nm. It appears feasible that various algal species can be identified by detection of their fluorescent signatures using a tunable laser as the excitation source. However, if one is concerned only with detection of chlorophyll a, the optimum excitation is approximately 600 ± 50 nm while detection is at 685 nm. An analysis of both calculations and laboratory results indicates that it should be feasible to measure chlorophyll a in concentrations as low as 1.0 mg/m^3 using a 100 kw peak pulsed laser from an altitude of 500 meters.

I. FLUORESCENCE OF CHLOROPHYLL AND OTHER PHOTO-SYNTHETIC PIGMENTS

I.A. Introduction

As an analytical tool, fluorescence has become one of the most important available to the analytic chemist, biologist, or biophysicist. As early as 1833, Brewster had observed the red fluorescence of chlorophyll extracted from green plants (this technique is still being used to demonstrate fluorescence to undergraduate chemistry students). In 1852 Stokes published a paper on fluorescence which included observations of fluorescence in quinine and chlorophyll among other chemicals.

More modern work appears to have begun in the 1930's. Purified chlorophyll a & b spectra were obtained in the 40's and the early 50's and immediately it appeared that these fluorescence techniques could be applied toward the study of photosynthesis.

An expansion in activity in this field occurred after high resolution spectrophotometric instruments became commercially available (about 1955).

Recently, use has been made of the laser as an excitation source. This development is referred to in Section I.D. Further possible advances in this area include high resolution spectroscopy, research on energy transfer times and stability of materials in high intensity

radiation.

I.B. Basic Principles of Fluorescence

The basic characteristic of fluorescence is that light of one wavelength is absorbed by a material and re-emitted at a different wavelength. Normally the emission, called fluorescence, is at a longer wavelength than the absorbed radiation and it is emitted within 10^{-8} to 10^{-9} seconds after the sample is exposed to the excitation light. The basic physical principles of this process are as follows. Consider a molecule in its state of lowest electronic energy. Absorption of an incident photon will raise the energy of the electronic system of the molecule. This excitation process takes place in times of $1/f$ where f is the frequency of the exciting radiation. Thus the excitation takes $\approx 10^{-15}$ sec. The excited state may persist for a considerably longer time ($\approx 10^{-8}$ sec) before collisions between the excited molecule and its neighbors take place, triggering a de-excitation process. There are several de-excitation processes which may occur. They include:

- 1) Resonance radiation
- 2) Rayleigh scattering
- 3) Raman scattering
- 4) Luminescence (Fluorescence and Phosphorescence)

Resonance radiation refers to the situation in which low pressure gasses absorb and re-emit the excitation light

before molecular interactions can occur. While resonance radiation is due to electronic excitations, Rayleigh scattering is a result of vibrational excitations and re-emissions from the molecule. In both resonance and Rayleigh scattering the emitted radiation is of same wavelength as the incident light, only distributed in all directions.

Raman scattering is very similar to Rayleigh scattering except that it occurs when energy is added to or subtracted from the vibrational energy of the molecule. In a given material these emissions of greater and lesser energy are emitted along with the excitation light. In liquids Raman bands are much weaker than Rayleigh scattering.

These scattering phenomena can affect the shape and size of fluorescence spectra but in general are of minor interest in a system which is only concerned with the fluorescence produced in a narrow band of wavelengths. Further, these effects are generally important only when the excitation and emission bands are close together.

The major de-excitation mechanism of interest to our current program is that of fluorescence.

A detailed analysis of fluorescence and phosphorescence requires an investigation of the quantum mechanical properties of the atoms and molecules of the material. Excellent reviews of fluorescence have been written by Becker (1969) and Hercules (1966).

A brief review of this analysis follows. All of the electrons in a molecule have a property called spin, the quantum number (S) of which is either $\pm 1/2$. In general, the total spin of all of the electrons in the molecule is zero since there are as many with positive spin $1/2$ as those with negative spin $- 1/2$. Further, the multiplicity of the molecule is defined as $(2S+1)$. If the molecule has paired spins, i.e., the total spin of the molecule is 0, the multiplicity is 1. From fundamental considerations, it can be shown that a molecule with multiplicity 1 has only 1 electronic level in the ground state. However, if the molecule does not have paired spins and S is not 0, the multiplicity is not 1 and a situation exists in which there are several closely spaced electronic energy levels. For instance, for the case $S=1$, there are three electronic energy levels. If $S=0$ the molecule is said to have a "singlet" electronic level, (multiplicity = 1) while in the latter case ($S=1$) it has a "triplet" level (multiplicity =3).

It has also been shown (Jablonski, 1935) that most molecules have excited electron levels that are either singlet or triplet, called F and P, respectively. In addition, it has been shown that transitions either by absorption or emission, from a singlet excited state to a singlet ground state are more probable than a transition

from a triplet to a singlet or vice versa. Fluorescence is the case where transitions take place between singlet levels. In fluorescence the molecular absorption takes place without destruction of the molecule and collisions do not significantly dissipate this energy and the re-emission will occur after a short time ($\approx 10^{-8}$ sec). The emitted light is of a longer wavelength (lower energy) than the absorbed energy, the difference being lost as heat in an accessory process.* Phosphorescence on the other hand describes the de-excitation of triplet levels to singlet ground states via excited singlet states. The lifetime of this process is much longer than for fluorescence (from μ secs to minutes) since the probability of transfer of energy from triplet to singlet excited states is quite small.

As a function of time, the fluorescent intensity (I) can be expressed

$$I = I_0 e^{-t/\tau}$$

where τ is the lifetime of the excited state and I_0 is the initial fluorescent amplitude. In addition there is a relationship between fluorescent intensity and concentration of fluorescent material which depends on the geometry of the

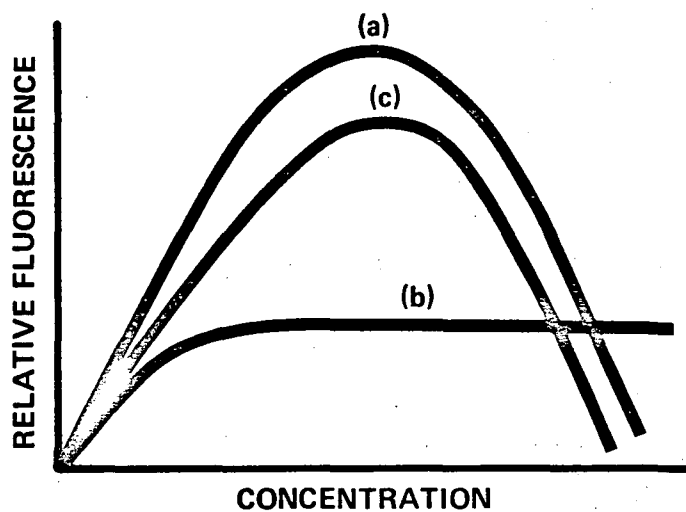
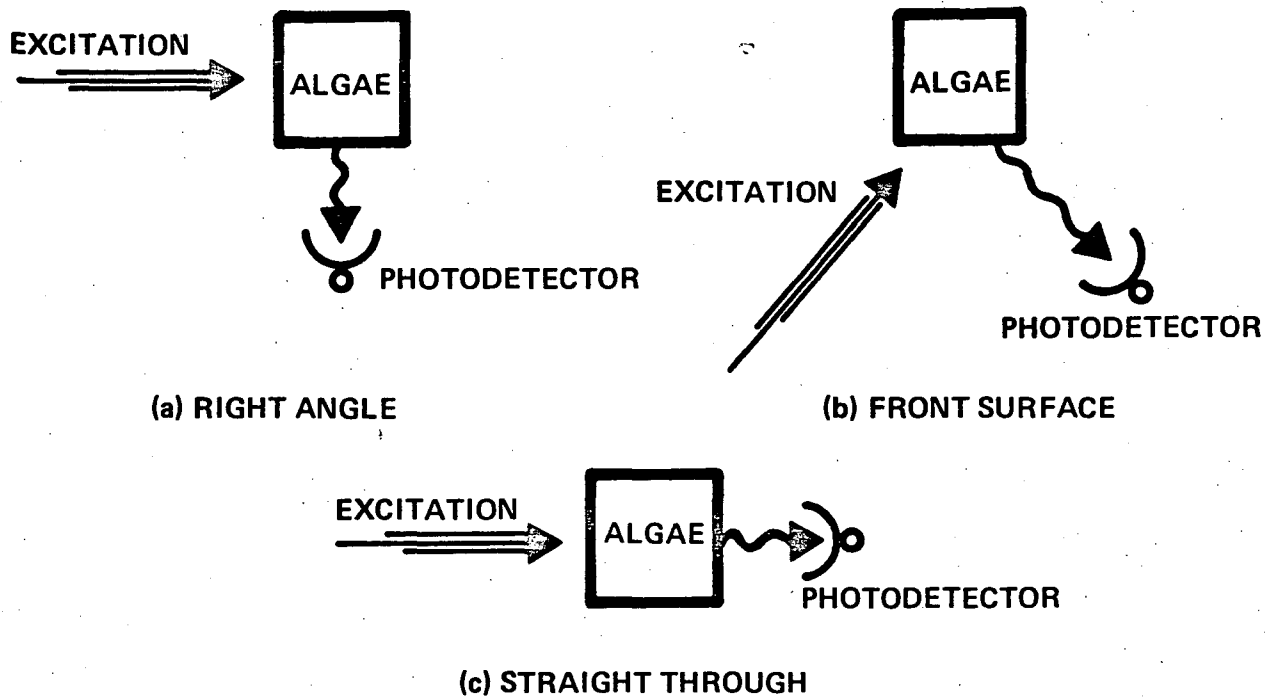
*The ratio of the number of emitted photons to the number of photons absorbed is commonly called the quantum efficiency of fluorescence.

experiment. Figure I-1 illustrates these relationships for right angle and surface geometries. In all of the measurement work done with spectrometers in this study, the right angle geometry is used. In an airborne system the front surface geometry would be applicable.

A feature of considerable interest in fluorescence is the so called mirror effect. This refers to the fact that the fluorescence spectrum of a material and its absorption spectrum usually appear to be reflected through a plane. This is illustrated in Fig. I-2. This is a widely observed phenomena and one that is not well understood. In addition, most polyatomic molecules (such as chlorophyll) have, in addition to the mirror absorption band, another substantial absorption band at shorter wavelengths. Light which is absorbed at the short wavelengths is re-emitted only in the long wavelength fluorescence band so that the shape of the emission band is independent of the excitation wavelength.

I.C. Fluorescence Spectra of Algal Pigments

Of the fluorescent pigments in algae, the most common is chlorophyll. Chlorophyll is a major factor in the ability of plants to utilize light for energy by photosynthesis. Its chemical structure is defined to be a metalloporphyrin which has magnesium as the metal component. There are several types of chlorophyll which



FLUORESCENT AMPLITUDE VS. CONCENTRATION FOR SEVERAL GEOMETRIES (AFTER UDENFRIEND)

FIGURE I-1

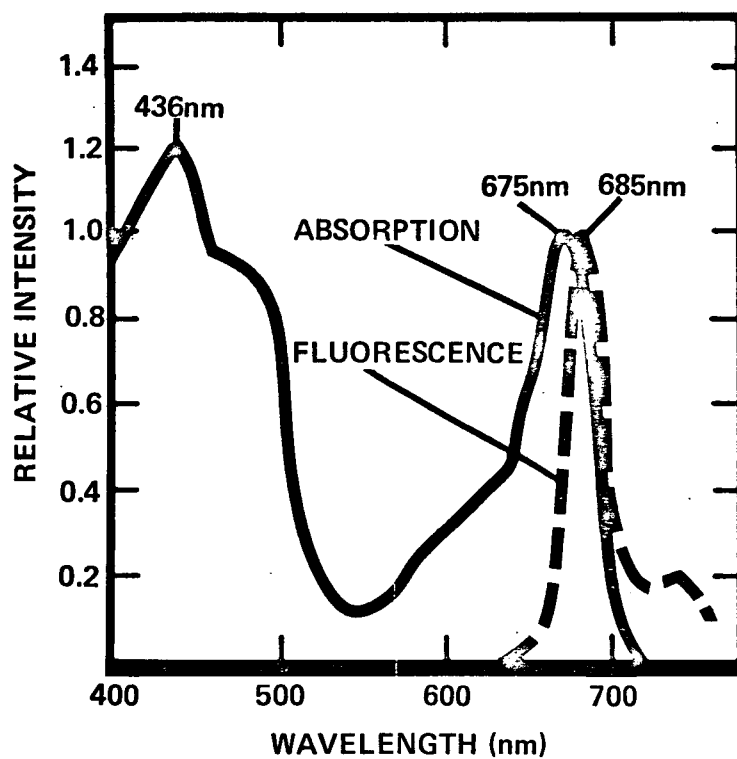


ILLUSTRATION OF THE MIRROR EFFECT.
IN ADDITION THE USUAL ULTRAVIOLET
ABSORPTION BAND IS SHOWN. THE
EXAMPLE USED HERE IS CHLOROPHYLL *a*

FIGURE 1-2

are only slightly different in chemical structure but which have different absorption and fluorescence spectra. These spectra are shown in Fig. I-3 a-d. The distribution of the various types of chlorophyll among the species of algae of interest are discussed in Appendix A. It should be noted chlorophylls b-d only fluoresce after extraction from algal cells.

Because chlorophyll a is an important factor in the conversion of light into energy for plants, it is not surprising that most of the light energy absorbed by live plants does not contribute to fluorescence. That is, the quantum efficiency of chlorophyll a is low. Values ranging from 0.15 to 2.8% have been reported (Vermuelen, et al, 1937 and Latimer et al, 1957) when measured in live plants. This value is somewhat larger when the chlorophyll is extracted from plants via an organic solvent, reaching values as high as 33% (Latimer, et al, 1957). In addition to chlorophyll, there are several other photosynthetic pigments whose absorption and fluorescence spectra are of interest.

The main accessories to photosynthesis are the carotenoids and the phycobilins, the latter existing only in algae. The carotenoids are yellow, orange, or red pigments which absorb light at wavelengths varying from 400 to 500 nm and are fat soluble. The carotenes do not fluoresce but transfer

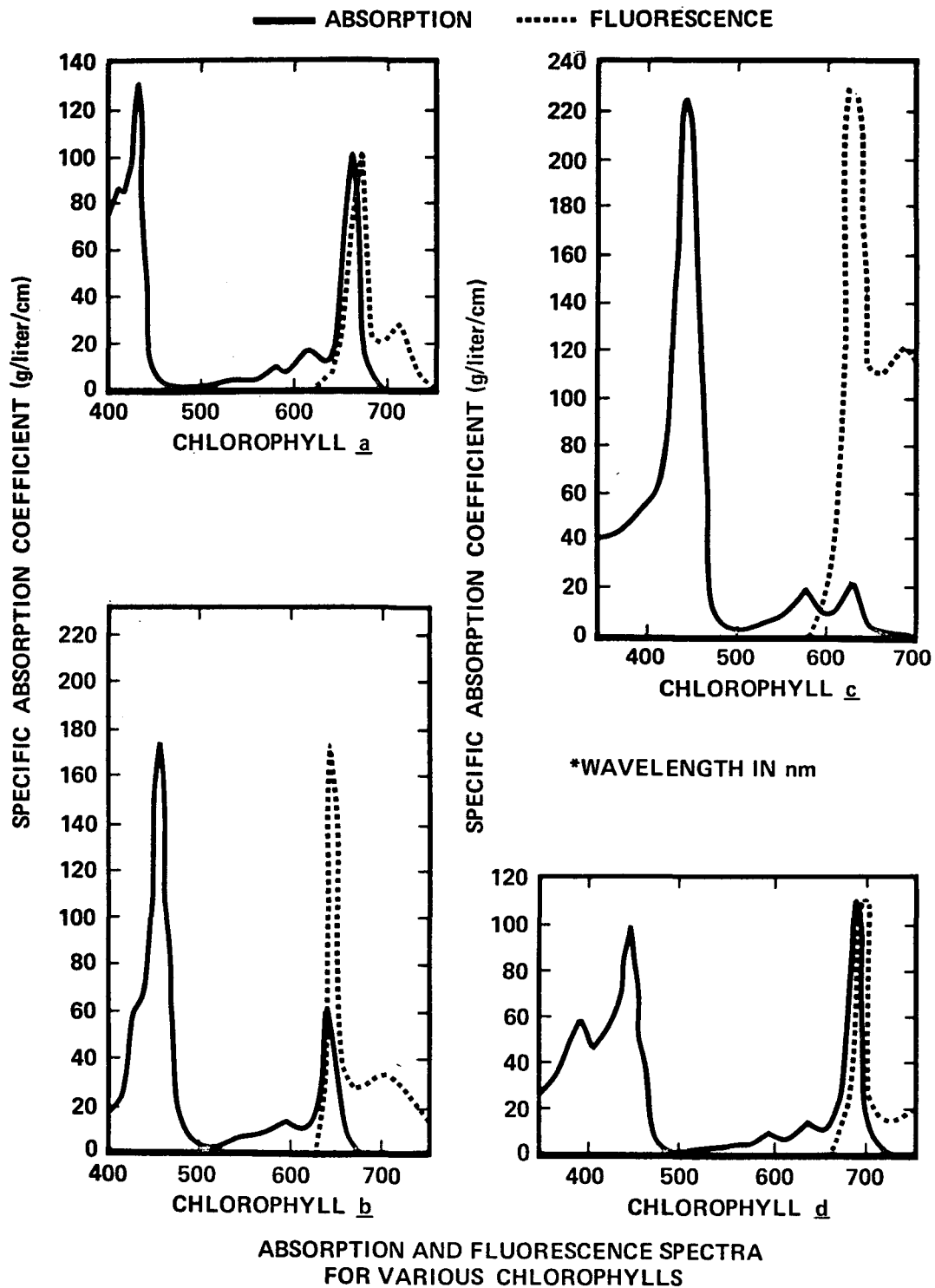


FIGURE I-3

their absorbed energy to chlorophyll a which may in turn fluoresce. The phycobilins are water soluble protein pigments and have their major absorption maxima in the region of 500 to 600 nm. The phycobilins fluoresce both in cells and after extraction. They also transfer absorbed energy to chlorophyll a with high efficiency.

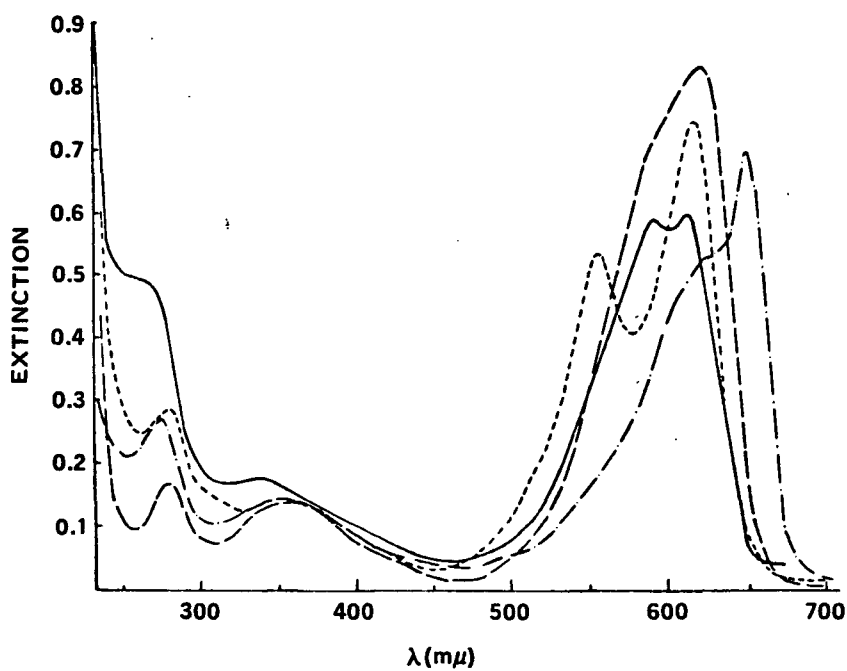
There are two main classes of phycobilins, i.e., phycoerythrin and phycocyanin. Various algae contain different types of the two classes. The three phycoerythrins which are frequently found in algae, and their absorption maxima are given below.

- a) R-phycoerythrin (495, 540, and 565 nm)
(found in red algae)
- b) B-phycoerythrin (540 and 565 nm)
(found in red algae)
- c) C-phycoerythrin (550 nm)
(found in blue-green algae)

The three phycocyanins which are frequently found in algae, along with their absorption maxima are:

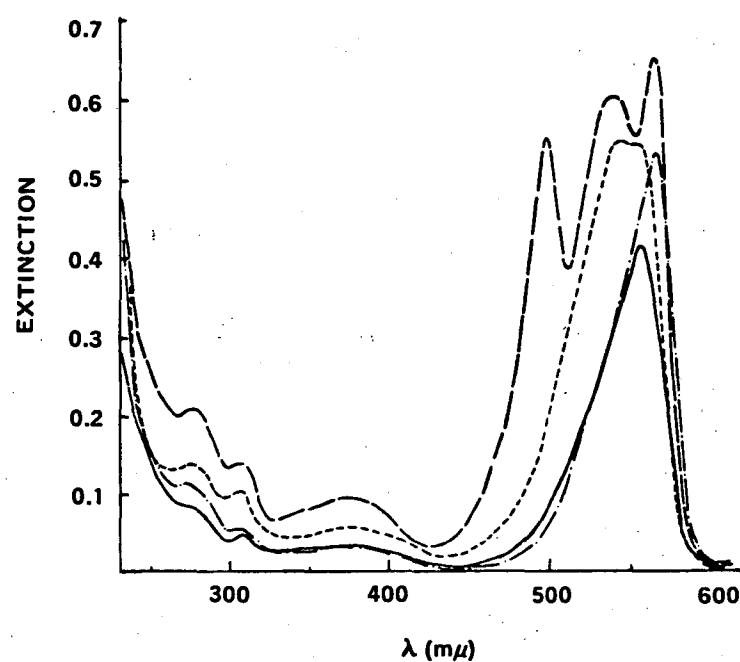
- a) C-phycocyanin (620 nm)
(found in blue-green algae)
- b) R-phycocyanin (550 and 620 nm)
(found in red algae)
- c) C-Allophycocyanin (650 nm)

Absorption spectra of the accessory pigments are shown in Figs. I-4, 5, and 6 after extraction from algal cells. The distribution of these pigments among the dominant classes of algae in the Chesapeake Bay is discussed in Appendix A. Given a knowledge of both the absorption and emission bands of these pigments one may project the appropriate excitation



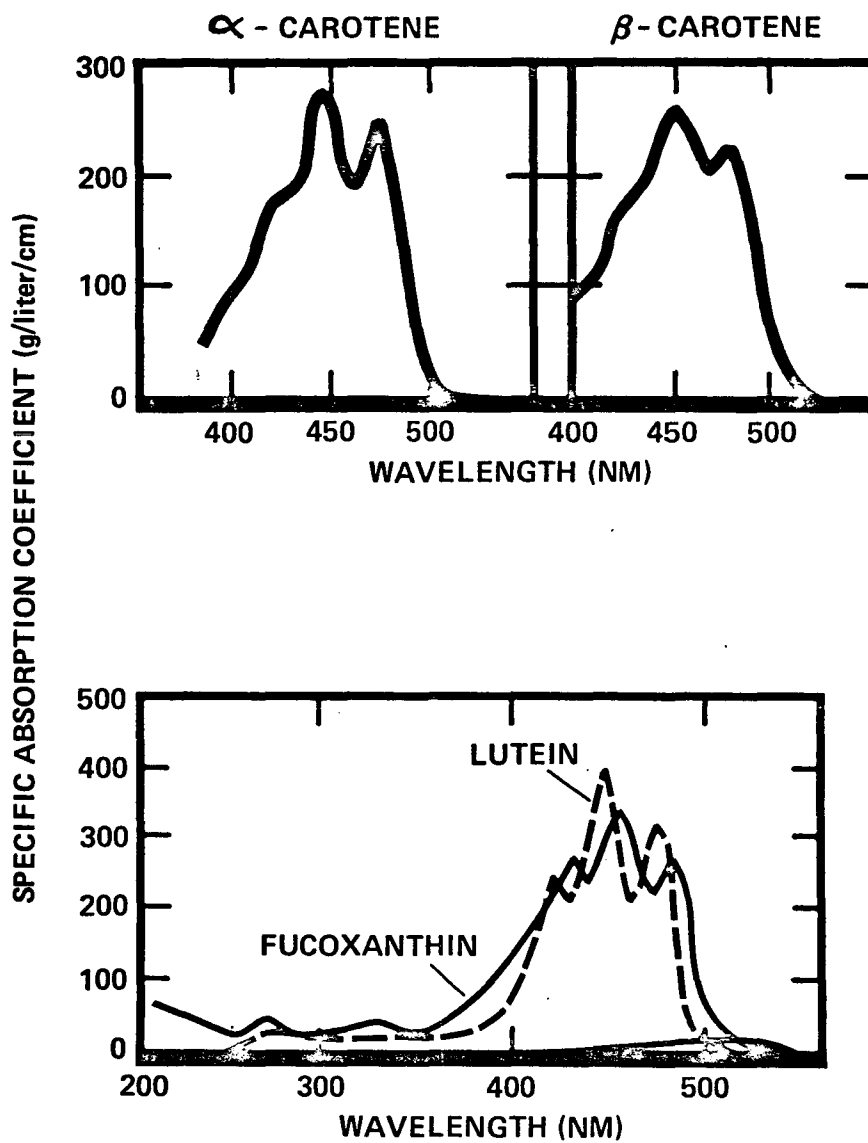
----- R-PHYCOERYANIN (RHODYMENIA PALMATA);
 - - - - - C-PHYCOERYANIN (LYNGBYA LAGERHEIMII);
 - · - · - ALLOPHYCOERYANIN (CERAMIUM RUBRUM);
 ——— CRYPTOMONAD PHYCOERYANIN (HEMISEIMIS VIRESCENS), PLYMOUTH
 STRAIN NO. 157)

FIGURE I-4 ABSORPTION SPECTRA OF AQUEOUS SOLUTIONS
 OF PHYCOERYANINS (pH 6-7) [AFTER C. H. H'EOCHA (1962)]



——— R-PHYCOERYTHRIN (CERAMIVM RUBRUM);
 - - - B-PHYCOERYTHRIN (PORPHYRIDIVM CRVENTVM);
 - · - C-PHYCOERYTHRIN (PHORMIDIUM PERSICINUM);
 ——— CRYPTOMONAD PHYCOERYTHRIN (HEMISDMIS RUFESCENS).

FIGURE I-5 ABSORPTION SPECTRA OF AQUEOUS SOLUTIONS OF PHYCOERYTHRINS (pH 6-7) [AFTER C. H. H'EOCHA (1962)]



ABSORPTION SPECTRA OF CAROTENES
(AFTER KARRER AND JUCKER)

FIGURE I-6

and emission bands of interest in an active remote sensing system for detection and identification of algae.

I.D. Basic Research Program

In light of the fluorescence properties of the photosynthetic pigments of algae, it has been suggested (Hickman and Moore, 1970) that remote sensing of the algae could be feasible if the fluorescence were generated by laser light.

This research program has been oriented toward examining the problems, capabilities and limitations of laser detection systems. Of course, the fluorescence properties of the algae are of primary significance. This feature of the problem has been considered in Section II, parts A-E. This work includes measurements of absorption and fluorescence spectra of various species of algae along with a determination of the fluorescence quantum efficiencies. Using this data, an extrapolation has been made to a remote airborne laser transmitter/detector system. A mathematical model for calculating the signal/noise ratio of a detector has been formulated. This model includes such parameters as background noise provided by the sun and skylight, geometric effects, laser power and detector field-of-view considerations, etc. This analysis is detailed completely in Section III along with its application to the system under consideration.

Based on the laboratory work and the mathematical analysis of the program, conclusions and recommendations have been made (Section IV) for optimizing the system capability and possible areas of further interest.

Appendices are included, covering a complete biological survey of the Chesapeake Bay, eye safety problems and recommendations, and an extensive literature survey.

II. MEASUREMENT PROGRAM/RESULTS

II.A. Measurement of Fluorescence Spectra

As a basic part of the general research program, the fluorescence spectra of various selected algae were made under a variety of conditions including various excitation wavelengths, concentrations, etc. These spectra were obtained using both a spectrophotofluorometer (xenon-arc lamp excitation) and a laser-scanning spectrometer system.

Laser Induced Fluorescence Measurements

The general layout of the experiment is shown in Fig. II-1. Excitation of the fluorescence in small samples (25 ml) of algae in solution was made using a nitrogen tunable dye laser (Avco Dial-A-Line) operating from 2 to 20 pulses per second. Each pulse had a duration of 3-5 nsec. and a peak pulse power in the order of 30K watts.

Focusing lenses were placed in the path of the beam so that upon entering the cuvette the beam was approximately 1 mm in diameter and had a pulse power of approximately 1000 watts. First surface mirrors were used to deflect the beam so that it entered the cuvette (and algal solution) from the top, producing a vertical, cylindrical column of fluorescence in the solution. The mirrors and lenses were positioned so that the fluorescing region was close to and directly in front of the entrance aperture of a Jarrell-Ash

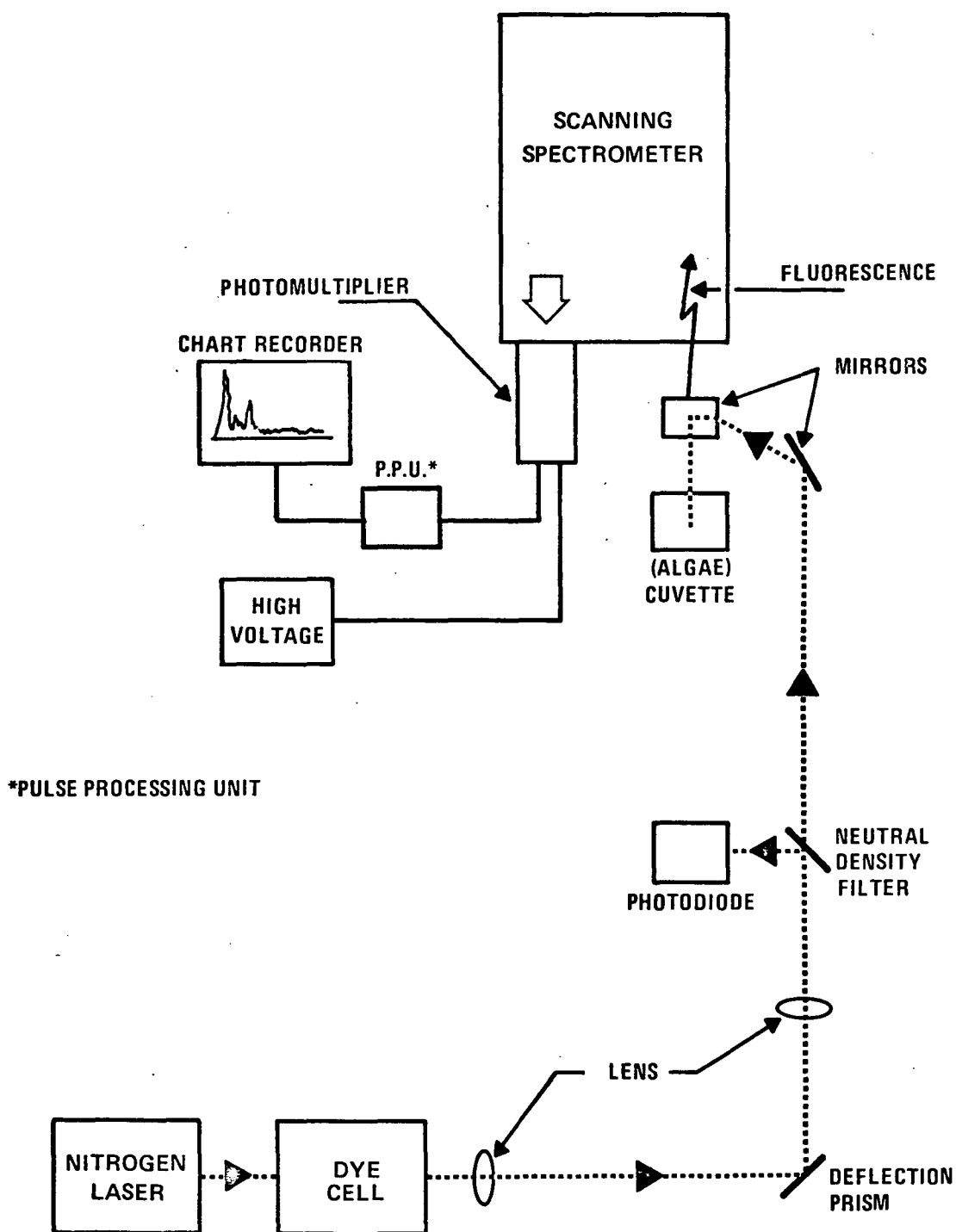
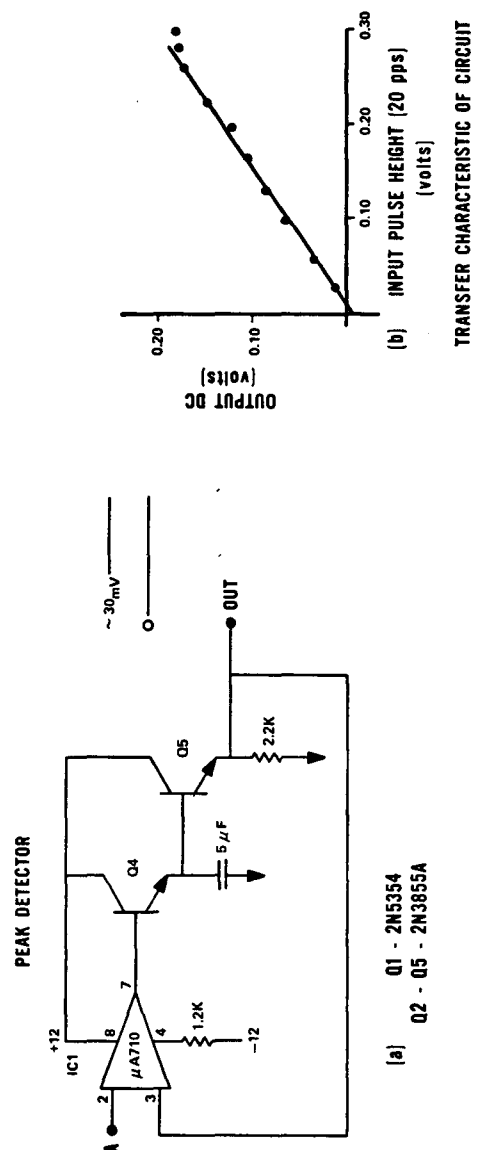
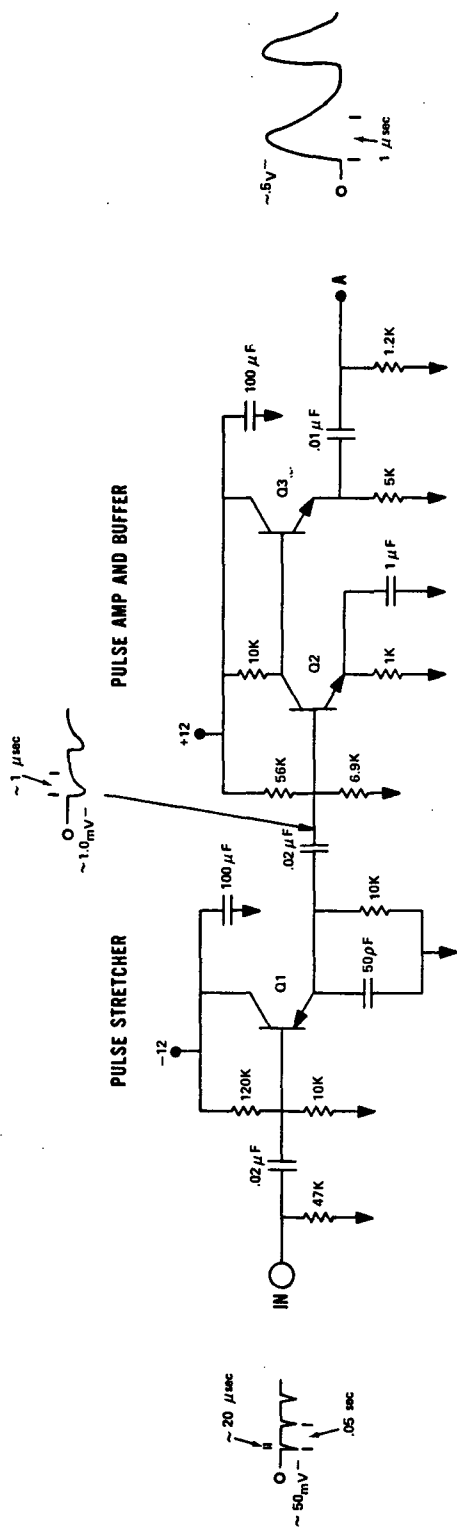


FIGURE II-1
A SCHEMATIC DIAGRAM OF THE EXPERIMENTAL SET-UP USED
FOR THE LASER STIMULATED FLUORESCENCE MEASUREMENTS

Czerny-Turner Scanning Spectrometer (Model #78-466). The output of the spectrometer was directed into a 12 stage photomultiplier tube (RCA Model #4459) which had an S-20 response curve. The tube was operated with an anode-cathode voltage of 1500 volts and had a sensitivity of 4×10^4 amps/watt of incident light. The output pulses from the photomultiplier tube (which were negative in voltage) were processed so that the fluorescence spectrum of the sample could be recorded on paper. For this reason a Pulse Processing Unit (PPU), shown in Fig. II-2 was designed and built by this laboratory. The circuit operates as follows. The pulses from the photomultiplier tube are stretched by transistor Q1. For input pulse durations of 20 nsec., the pulse duration at the base of the pulse amplifier Q2 is 1μsec. Q3 provides unity gain and buffers the output of the pulse amplifier and drives the input of the high speed comparator, IC1. The output of the comparator turns on the current driver, Q4, which charges up the storage capacitor when point 2 of the IC1 exceeds the voltage at point 3. The voltage at point 3 is determined by the unity gain buffer from the storage capacitor. In this way the dc output level is directly proportional to the input pulse heights. The transfer characteristic of the circuit is also shown in Fig. II-2.



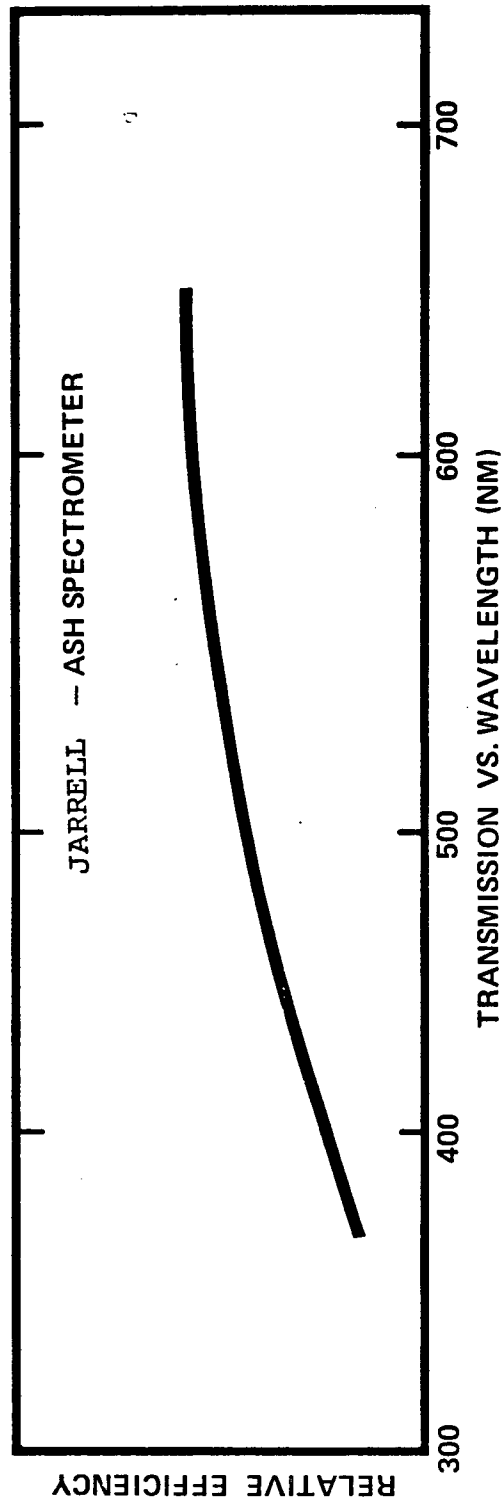
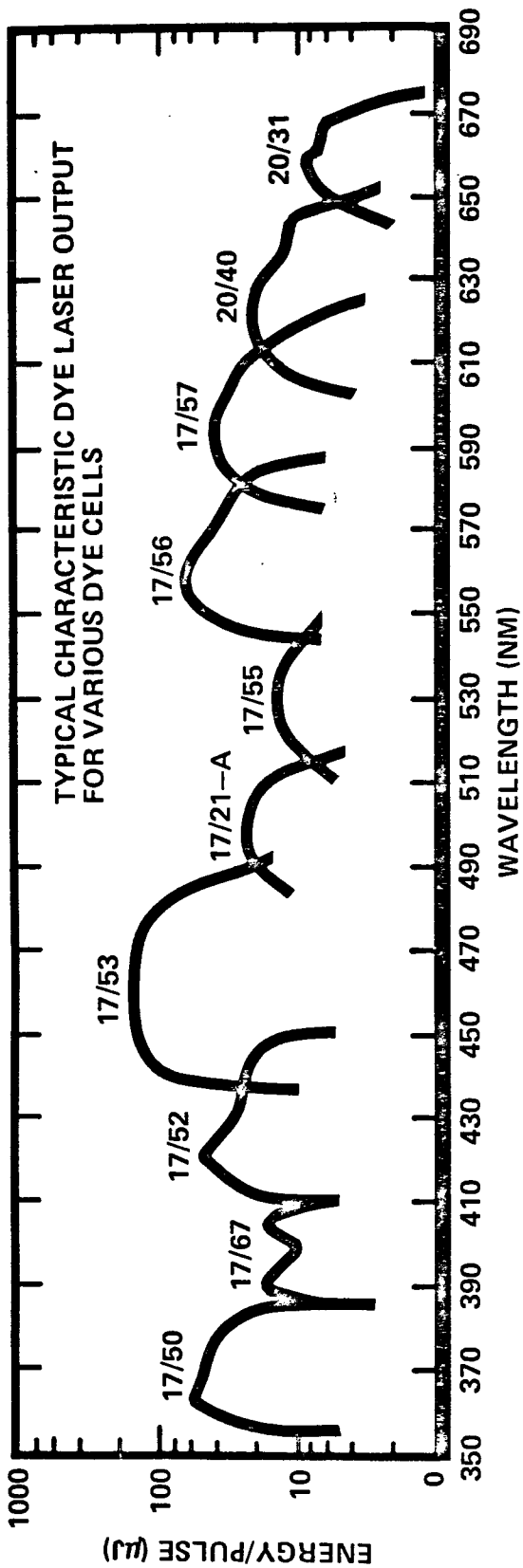
PULSE PROCESSING UNIT
FIGURE II-2

Using this system, the fluorescence spectrum of a particular sample was measured over the entire optical spectrum for a given excitation wavelength. The excitation wavelength was then changed and the emission spectrum remeasured. This technique was used to measure the excitation/fluorescence spectra over the optical spectrum. All spectra were corrected for several system parameters including phototube response, laser power, and spectrometer grating efficiency. For example, the output power of the laser varies as the output wavelength of the laser is changed. To correct for this, each fluorescence spectrum was divided by the output power of the laser (which was constantly monitored). Figure II-3 illustrates the laser output and grating efficiency as a function of wavelength.

The spectra generated by this system are presented in Fig. II-4 a, b, c, and d as points superimposed on the continuous spectra obtained from the spectrophotofluorometer. The spectra have been obtained for the four different algal types; *Chlorella pyrenoidosa*, *Porphyridium cruentum*, *Agmenellum quadruplicatum*, and *Chlamydomonas reinhardt*. Figure II-5 is a composite curve showing the four spectra.

Spectrophotofluorometer Fluorescence Measurements

A set of experiments similar to those described above were performed using a conventional spectrophotofluorometer



CHARACTERISTICS OF LASER SPECTROMETER

FIGURE II-3

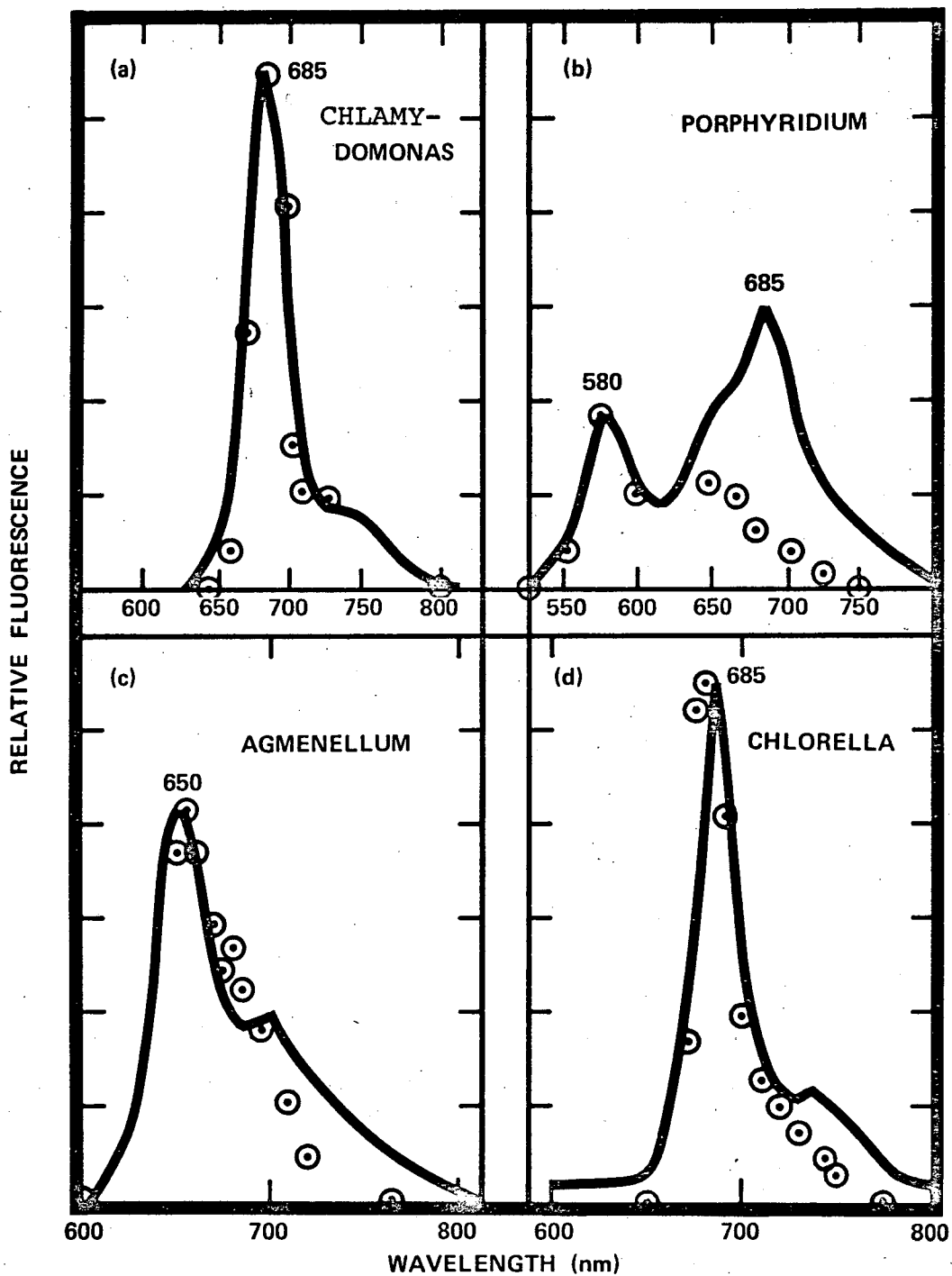
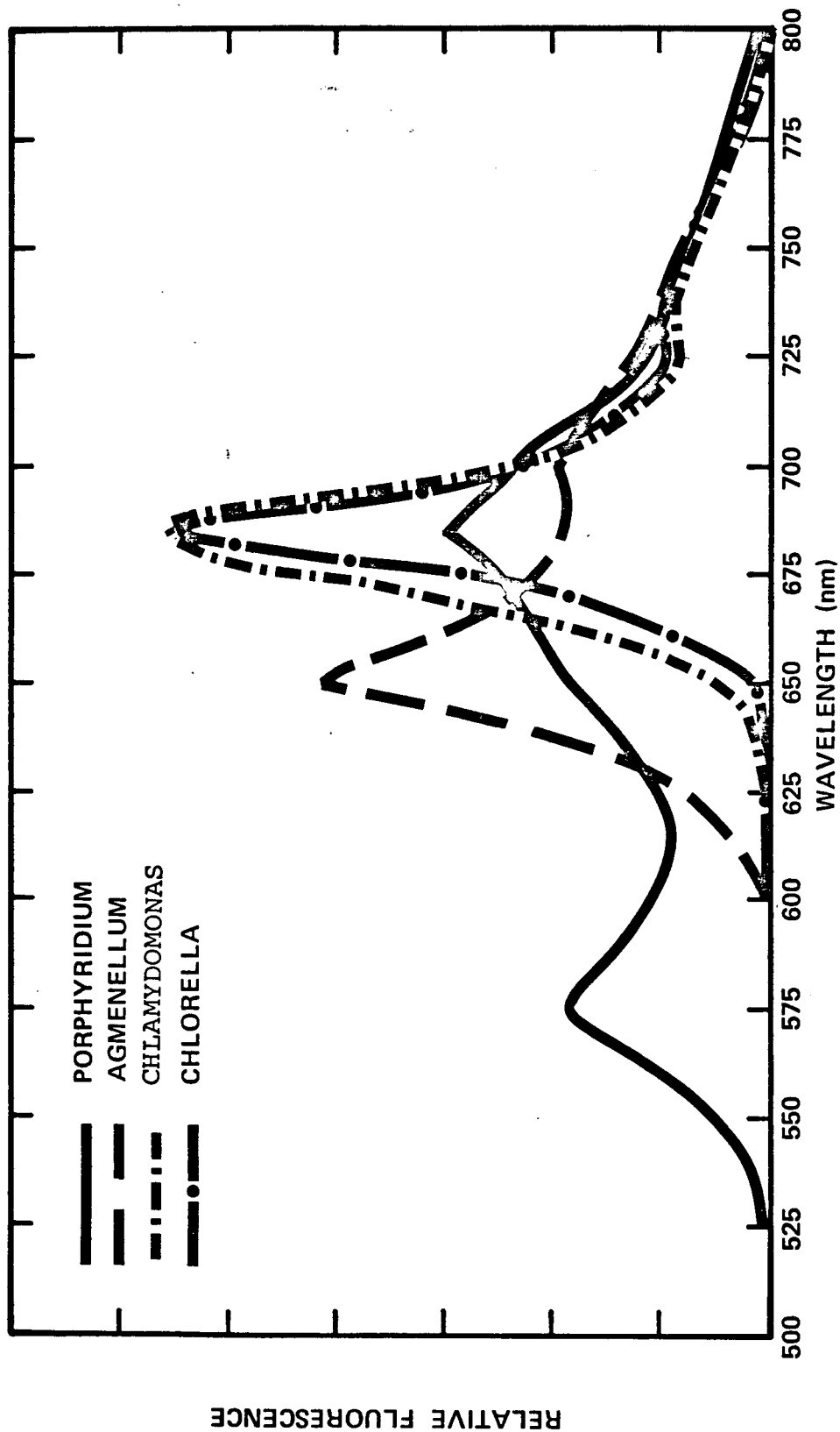


FIGURE II-4 FLUORESCENT SPECTRA OF VARIOUS SPECIES OF ALGAE .
(CIRCLES ARE LASER GENERATED DATA WHILE THE LINES
INDICATE DATA OBTAINED FROM A SPECTROPHOTOFUOROMETER)



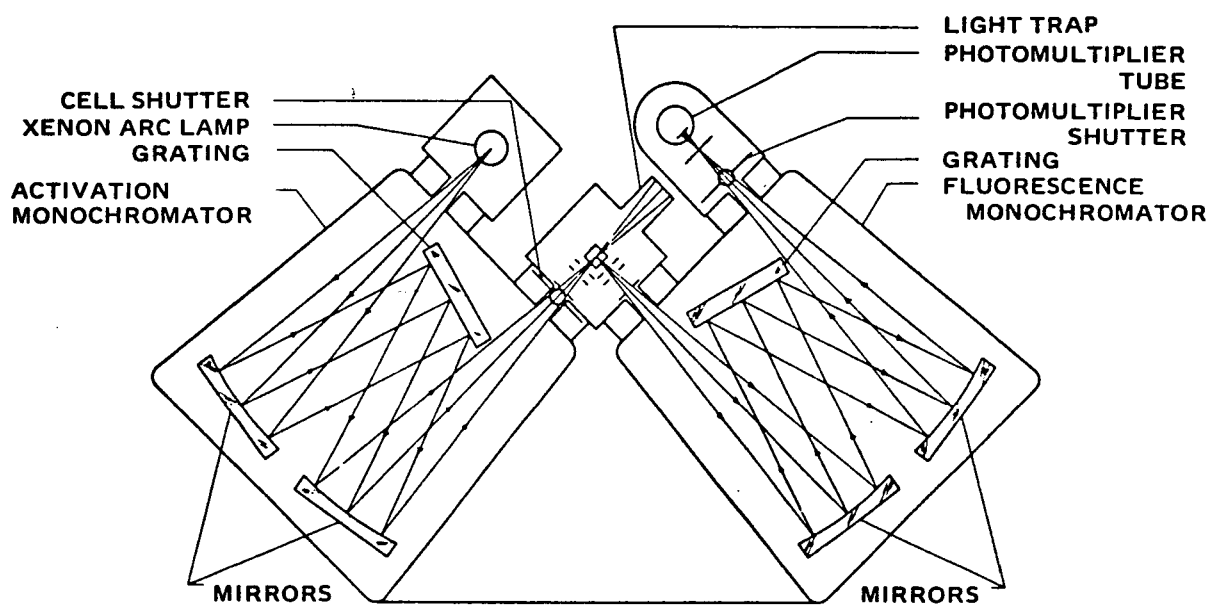
FLUORESCENCE SPECTRA OF FOUR SPECIES OF ALGAE

FIGURE II-5

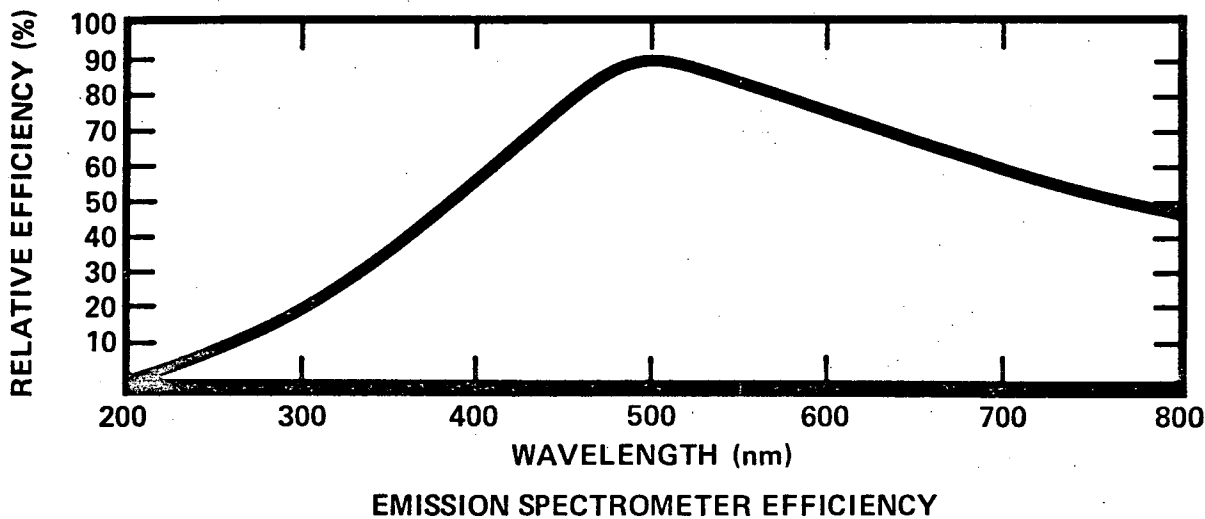
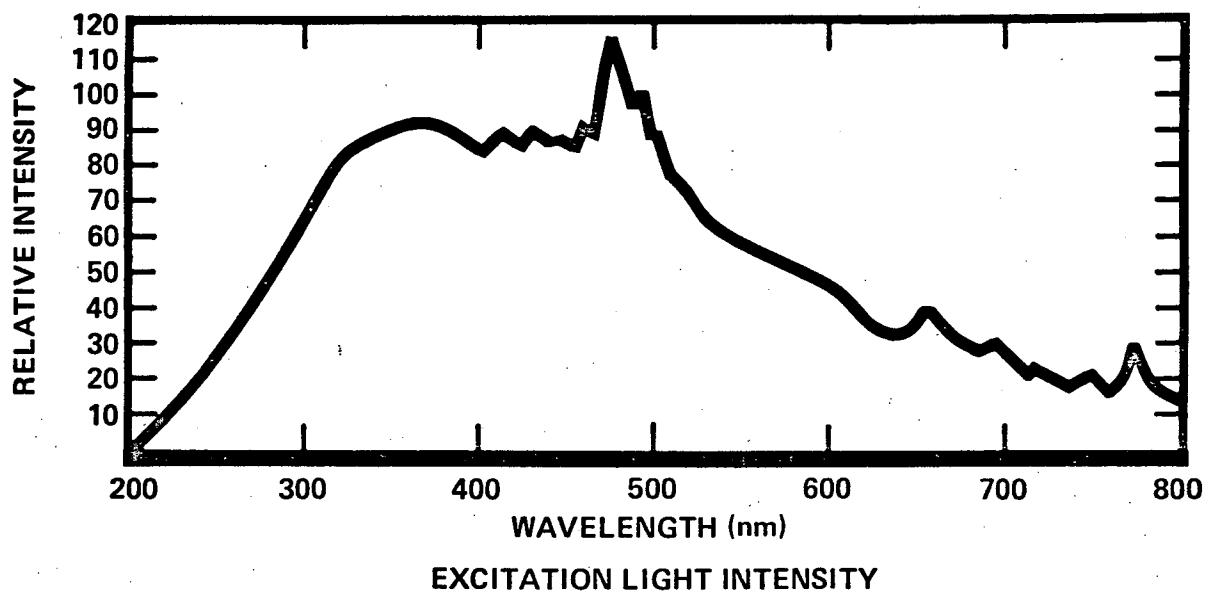
(AMINCO-BOWMAN). The source of excitation was a xenon arc lamp. Optical layout of this instrument is shown in Fig. II-6. The lamp emission is directed by a mirror to the excitation grating then to another mirror which directs the light toward the sample area. At this point a slit was inserted to limit the light striking the sample to a small percentage of the spectral output of the grating. Clearly the bandwidth of the excitation light depended on the width of this slit, which was generally set at 2 mm. The resultant resolution was 11 nm. The fluorescence generated by this optical system was viewed at right angles by a mirror and grating system of the same type and the resultant light detected by a photomultiplier (RCA 31025C). The grating in the emission monochromator had a 500 mm blaze. Figure II-7 a) shows the intensity of the lamp measured at the sample position and Figure II-7 b) shows the relative combined efficiency of the grating and photomultiplier. The output of the photomultiplier was connected to a photometer (current to voltage converter) and to an XY recorder. The X axis of this recorder was driven by a voltage proportional to the grating setting. Both gratings could be moved either manually or by a variable rate motor driven system.

Spectra taken with this instrument are shown in Figs. II-4 and II-5.

As in the case of the laser spectrometer, the results obtained from the SPF were corrected for relative excitation



**FIGURE II-6 SPECTROPHOTOFLUOROMETER
(AMINCO-BOWMAN)**



CHARACTERISTICS OF SPECTROPHOTOFUOROMETER

FIGURE II-7

power, grating efficiency, and photomultiplier sensitivity as a function of wavelength.

In both systems, quartz cuvettes were used to minimize fluorescence of the container and to allow UV excitation. In cases where the fluorescent component exists in trace amounts, spectroscopists are forced to use spectroscopic grade solvents and double distilled water. This was not a significant factor in these measurements and the dilutions of the original cell concentrations were done with distilled water. The concentrations were high enough to insure that impurities in the water did not have a significant effect.

Cuvettes were cleaned regularly in Calgonite and rinsed thoroughly before use. At regular intervals, the cuvettes were filled with fuming nitric acid and allowed to stand for extended periods. This removed the particularly stubborn stains from chlorophyll and other chemicals being studied in the instrument.

Occasionally calibration of the monochromator settings of both spectrometers was necessary. This was done by using two wavelength references, a low pressure mercury vapor lamp, which has a series of narrow emission lines, and a He-Ne laser which has a single emission line at 632 nm.

II.B. Measurement of Absorption and Action Spectra of Algae

An important aspect of the measurement program was to obtain the absorption spectra for the algal species for a variety of concentrations. These measurements were made

on a Cary 14 dual beam spectrometer.* This instrument is capable of making high resolution absorption measurements as well as suppressing variations in the lamp output.

Some distinction should be made between the absorption spectra and the excitation spectra. Excitation spectra illustrate the effectiveness of various wavelengths at generating a particular band (usually the 685 nm peak) of fluorescence. The absorption spectrum, on the other hand, shows the total absorption at various wavelengths, regardless of whether the absorbed light generates a fluorescent signal. The difference between the two curves represents the amount of energy which is absorbed and is not transferred to creation of chlorophyll a fluorescence. In general, the two curves overlap at longer wavelengths but show a discrepancy in the near uv. Figure II-8 shows the excitation and absorption spectra of Agmenellum, Chlorella, Chlamydomonas, and Porphyridium respectively.

All of the spectra presented here have been corrected for any system parameter which varies with wavelength.

In addition to identifying the various absorptive pigments in the algae, these measurements provided the optical density data necessary for the determination of the quantum

*These results were graciously provided by Dr. Elizabeth Gantt of the Smithsonian Institution Radiation Biology Laboratory.

⊙ LASER EXCITATION DATA

— SPF EXCITATION DATA

..... ABSORPTION DATA

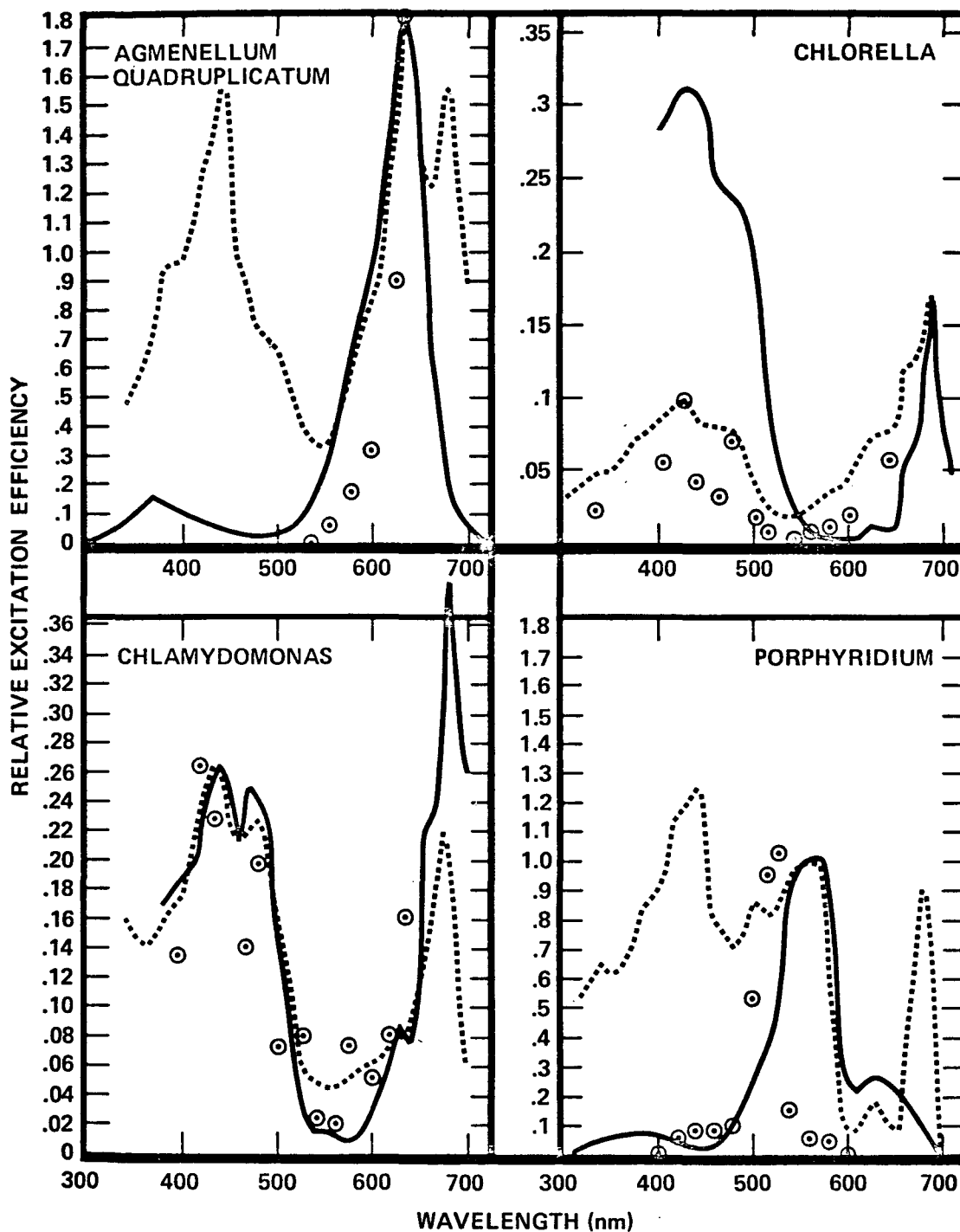


FIGURE II-8
ABSORPTION AND EXCITATION SPECTRA OF ALGAL SPECIES

efficiency.

II.C. Measurement of Quantum Efficiency

One of the most important parameters used in the mathematical analysis is the quantum efficiency for the fluorescence of the photosynthetic pigments in the plankton.

The quantum efficiency q is defined as the number of quanta emitted at the fluorescence wavelength divided by the number of quanta absorbed by the sample.

In general, measurement of absorption properties of solutions is relatively simple since the optics of the system may be configured so that the beam of light used for the measurement is well collimated and its intensity easily measured. However, the fluorescence is emitted in all directions and measurement of the total intensity is quite difficult. This type of measurement has been made, however, for several organic dyes in our laboratory and these are used to calibrate the quantum efficiency of the algae. This technique has been described by Parker and Rees (1960) and is reviewed here. If the fluorescence spectrum of a substance whose q is known is measured in a spectrometer which is also used for the generation of the fluorescence spectrum of a substance in question, the unknown q is given by

$$q_u = q_r \frac{F_u}{F_r} \frac{O.D._r}{O.D._u} \frac{I_r}{I_u} \frac{\lambda_{re}}{\lambda_{ue}} \left(\frac{\lambda_{uf}}{\lambda_{rf}} \right)^2$$

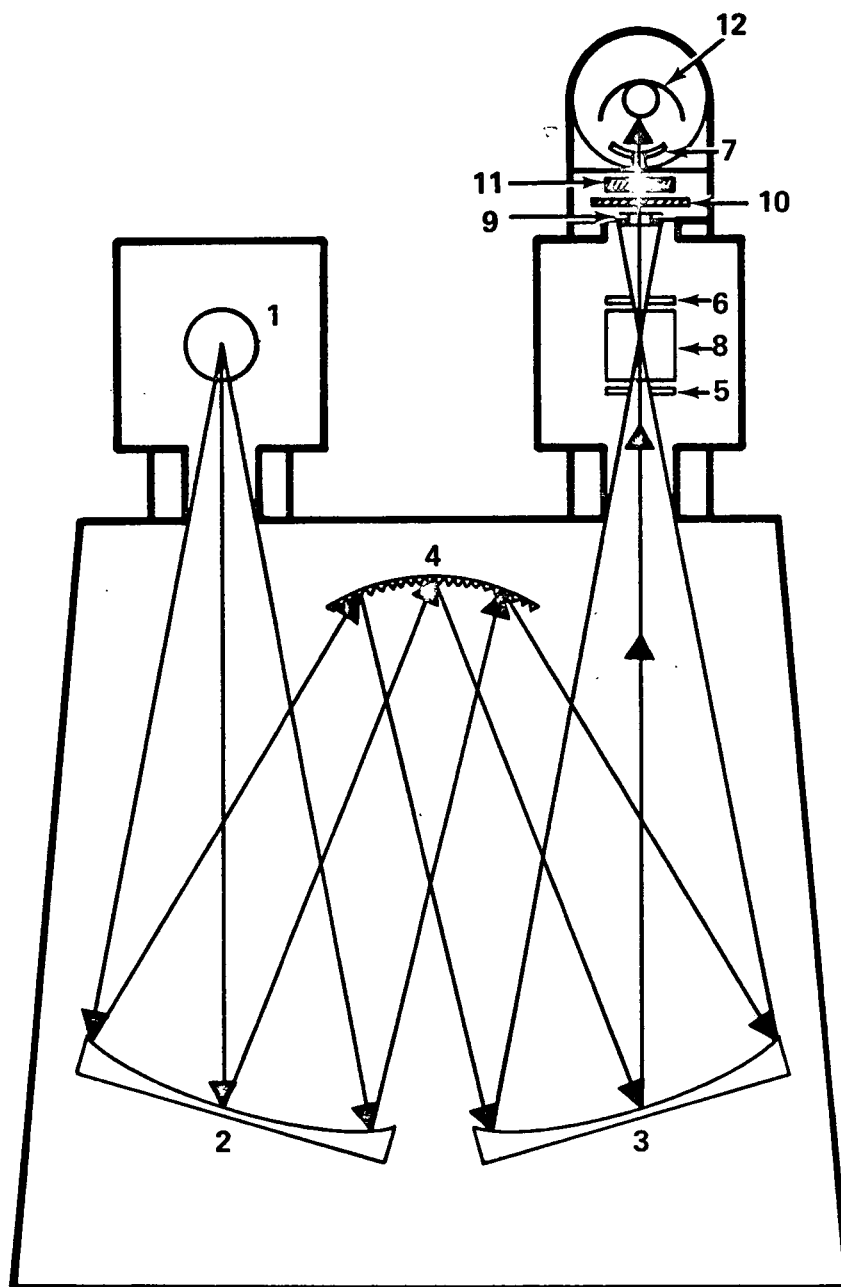
This is a modified form of an equation given by Udenfriend (1962). The subscript u refers to the unknown sample while the subscript r refers to the reference material. F is the area under the fluorescence curve which must be corrected for phototube response and grating efficiency. The optical density is designated by (O.D.) of the solution while I is the intensity of the light used to excite the fluorescence. λ_E is the excitation wavelength and λ_F is the peak fluorescence wavelength. The measurement of the optical density is made relative to the transmission of distilled water.

Several standards have been suggested in the literature. Two of the most common are quinine sulfate in .1NH₂SO₄ (q=.55) and Rhodamine B in Ethanol (q=.97). For the present work, Rhodamine dissolved to 1 part per million in Ethanol was chosen as the standard.

The AMINCO-BOWMAN Spectrophotofluorometer (SPF) equipped with an RCA 31025C phototube was used for these measurements. As described in Section II-A, the fluorescence spectra of both Rhodamine and various species of algae were measured and their spectra appropriately corrected. The excitation wavelength was chosen to be 337 nm. As can be seen only slight spectral corrections are required since the emission grating-phototube combination is relatively flat over the range of wavelengths of interest. In

addition, for smaller fluorescence emission bandwidths the relative change in system efficiency is smaller and thus the appropriate correction is smaller. Once the fluorescence measurements were obtained, the system was converted to the transmission measuring mode so that the optical densities could be measured using distilled water as a reference. A schematic diagram of the SPF used for transmission measurements is shown in Figure II-9.

This data was then used to compute \underline{q} for several values of algal cell concentration so that an extrapolation to zero concentration could be made. This was necessary because of self-absorption of the fluorescence light. Table II-1 presents data on the dyes which were obtained during this study along with the measured \underline{q} values for various species of algae extrapolated to zero concentration. Figure II-10 shows the quantum efficiency as a function of concentration (O.D.) for excitation at 337 nm for the various algal species. Included in Table II-1 are the values of \underline{q} for the 10 nm band centered at 685 nm. This 10 nm band simulates the filtering which is required of an operational system. This filter is chosen to coincide with the maximum algal fluorescence (produced by chlorophyll a) which peaks at 685 nm. The significance of this \underline{q} value is that it represents the actual quantum efficiency for the generation



- | | |
|-------------------|----------------------------|
| 1. XENON ARC LAMP | 7. SLIT |
| 2. } MIRRORS | 8. CUVETTE |
| 3. } | 9. PHOTOMULTIPLIER SHUTTER |
| 4. GRATING | 10. NEUTRAL DENSITY FILTER |
| 5. } SLITS | 11. DIFFUSING PLATE |
| 6. } | 12. PHOTOMULTIPLIER TUBE |

SETUP OF SPECTROFLUOROMETER FOR TRANSMISSION MEASUREMENTS

FIGURE II-9

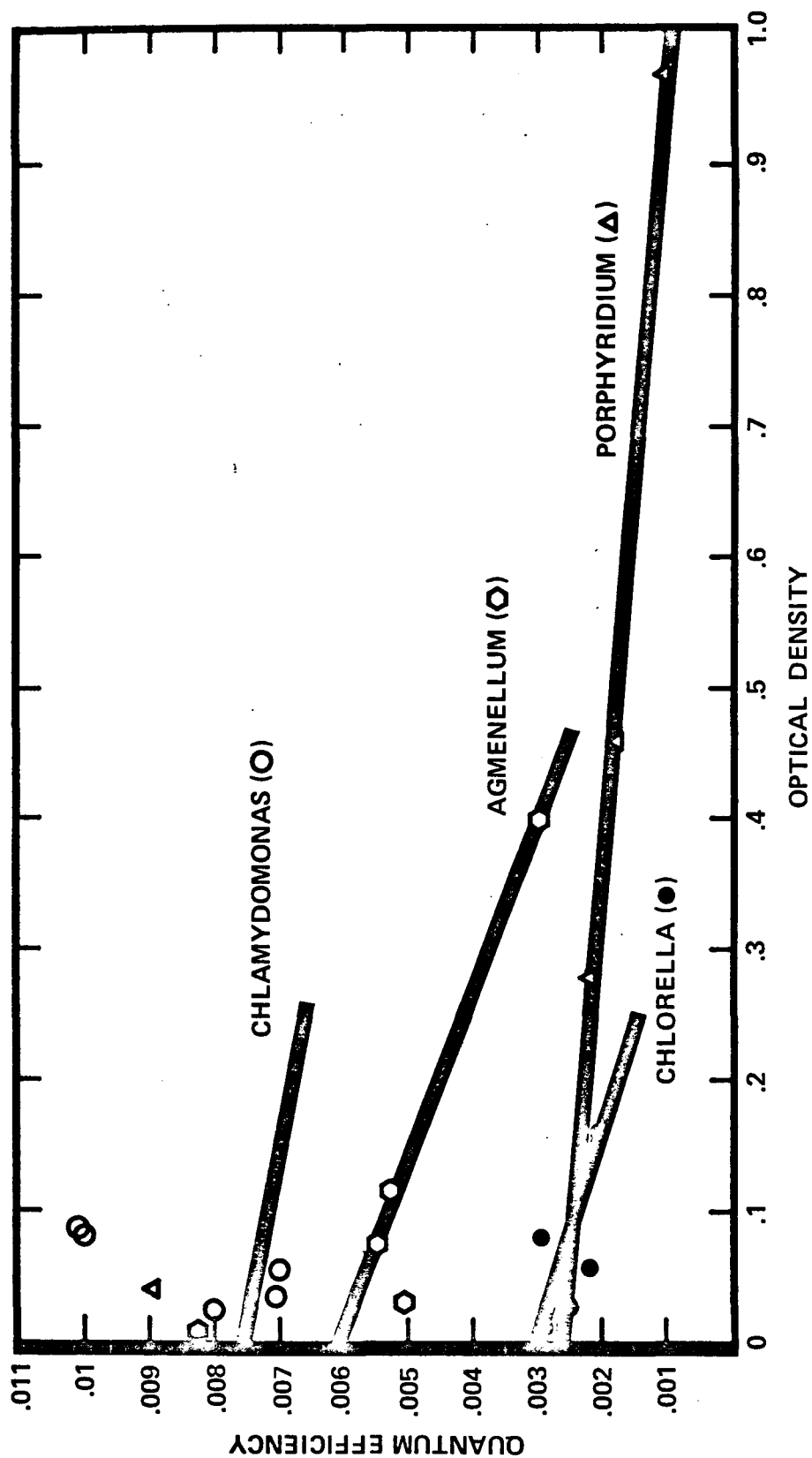
TABLE II-1

Species	Excitation	q(%)	q of 10 nm band at 685(%)	Ref.
Agmenellum	337	.6	.03	1
Chlorella	337	.291	.063	1
Chlorella	436	2.7		2
Chlorella	436	1.7-2.0		2
Chlorella	436	.15-.3		3
Navicula min.	436	2.8		2
Nitzschia sp.	436	.25		4
Synechocystis sp.	436	1.5		2
Porphyridium	337	.26	.014	1
Chlamydomonas Rein.	337	.7	.15	

Dyes

Rhodamine B	366	.68		1
Rhodamine B	366	.73		5
Acridine Orange	366	.53		1
Acridine Orange	366	.46		5

- 1 - Friedman and Hickman (1972)
 2 - Latimer Bannister Rabinowitch (1957)
 3 - Vermeulen, Wassink and Reman (1937)
 4 - Wassink and Kersten (1944)
 5 - C. A. Parker (1968)



QUANTUM EFFICIENCY OF VARIOUS ALGAL SPECIES AS
A FUNCTION OF CONCENTRATION (OPTICAL DENSITY)

FIGURE II-10

of that portion of the fluorescence spectra that will be detected by an operational system.

It should be noted in Table I that the q values vary from specie to specie. This is a result of the fact that a specie like Agmenellum quadruplicatum which has a predominant phycocyanin fluorescence has a fairly low fluorescence output at 685 nm. However those species whose fluorescence is mostly from Chlorophyll a have a relatively high q value at 685 nm.

II.D. Simulated Remote Sensing Measurements

Simulated remote sensing experiments were performed in an effort to make simple tests of the basic assumptions about an operational system.

These measurements were performed using the laser as the excitation source and a photomultiplier spectrally filtered as the detector. Two basic measurements were made. First, the dependence of the detected signal on concentration of algae was measured in small water tank. The configuration of the experiment is shown in Fig. II-11 a). The experiments were done on the algal specie Agmenellum. A 10 nm interference filter, centered at 680 nm was chosen to allow detection of the chlorophyll fluorescence by the photodetector, but block the excitation wavelength at 610 nm.

Figure II-11 b) shows the results of this study, with the detector 1 meter above the surface of the water.

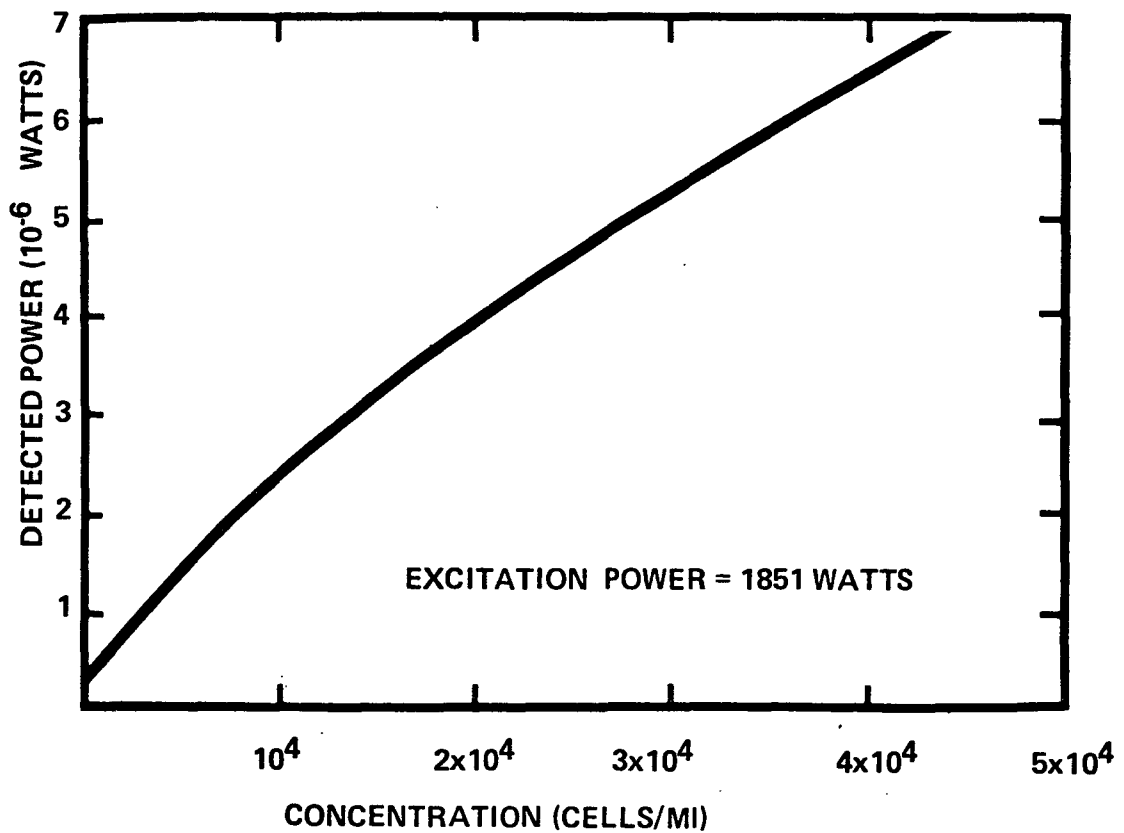
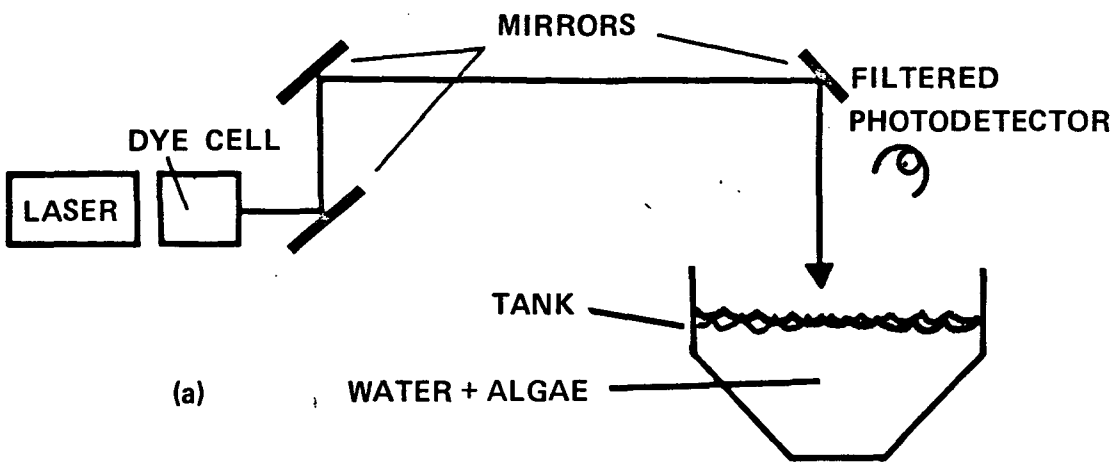


FIGURE II-11
FLUORESCENCE VS CONCENTRATION (THE DETECTOR
LOCATED 1 METER ABOVE THE WATER.)

Figure 11-12 shows the result of a similar experiment.

In this latter case the algae was contained in a plexiglass container located 12 meters from the laser/detector.

The dependence of the detected fluorescence signal on distance was determined by fixing the algal cell concentration in the container and measuring the signal as a function of distance between the algae and laser/receiver. The results of this experiment are given in Fig. II-13.

It should be noted that for small concentrations the signal is proportional to the concentration of cells. For a relatively thin water sample containing algae, the majority of the exciting laser beam is transmitted through the sample containing the algae and thereby does not contribute to the fluorescence signal. This condition would not exist in a large body of water. In the latter case the entire beam would be absorbed, generating fluorescence in the entire algal water volume. The fluorescence generated deep in the water would of course be reabsorbed more than that generated near the surface. The observed fluorescence is therefore a complicated factor of the environment.

As expected, the distance dependence measurements indicate a fluorescence signal which falls off approximately as $1/R^2$. A more detailed analysis of the problem shown

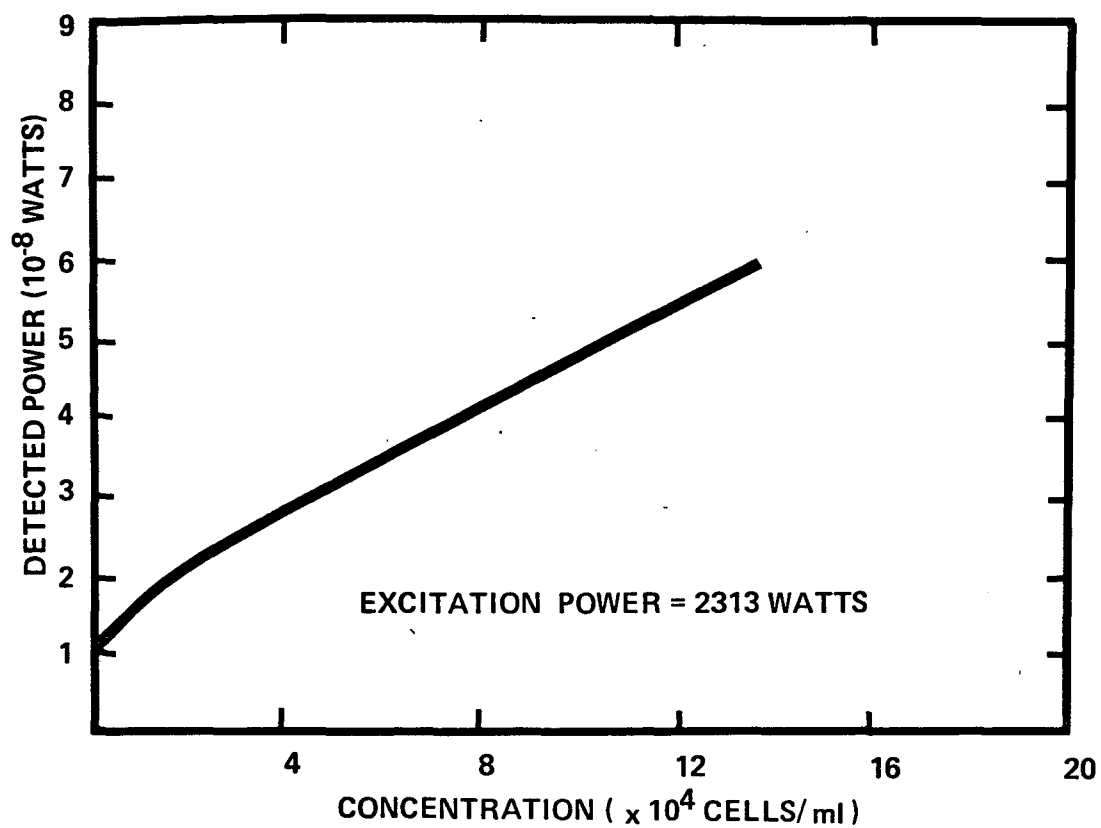
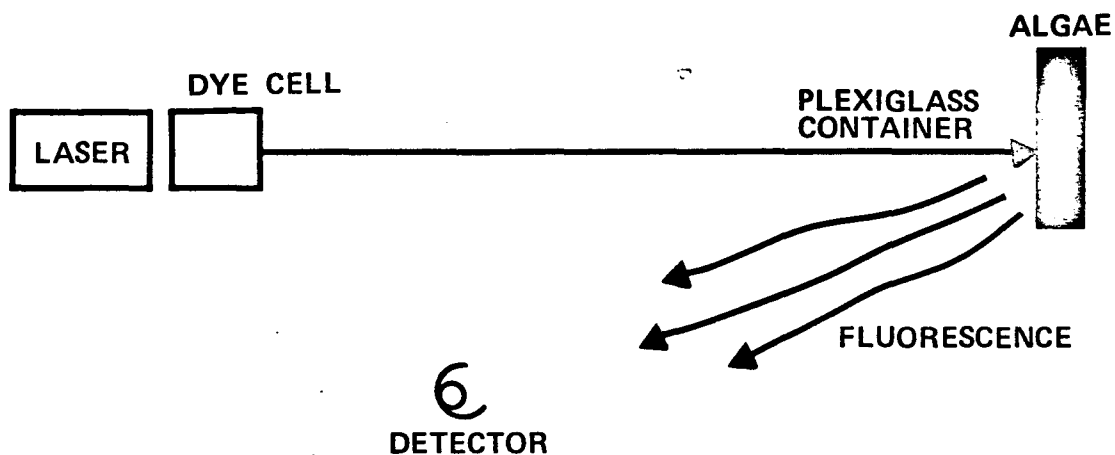
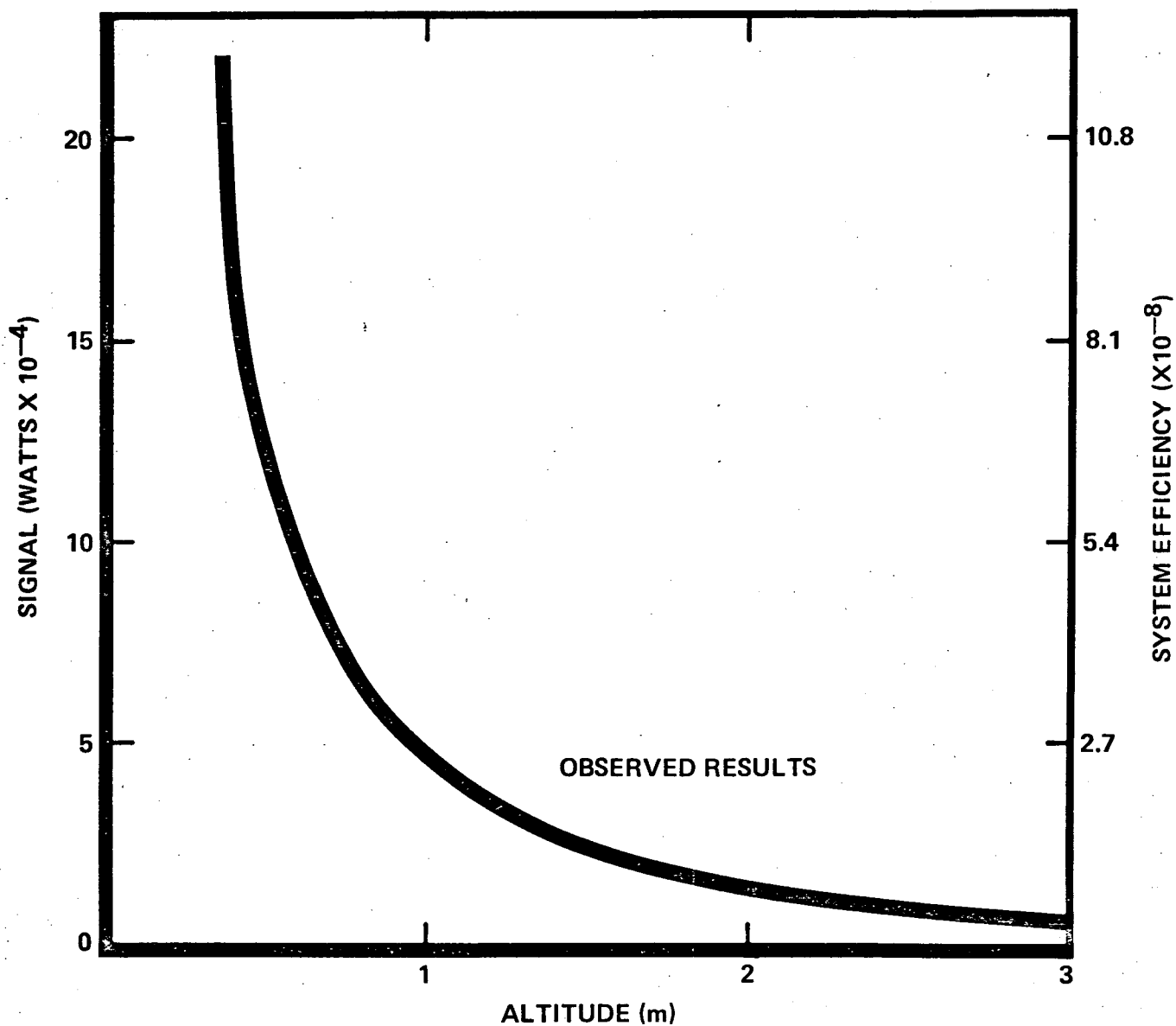


FIGURE II-12
FLUORESCENCE VS CONCENTRATION (THE DETECTOR LOCATED
12 METERS FROM THE SAMPLE.)



DEPENDENCE OF SIGNAL ON DISTANCE
OF SAMPLE FROM LASER RECEIVER

FIGURE II-13

in Section III shows a more complicated dependence of the signal on R. However, for most cases, the $1/R^2$ term is predominant.

It should also be noted that a system efficiency value has been included in the results shown in Fig. II-13. This efficiency refers to the ratio of the detected power to the primary laser power. Computations presented later indicate that this efficiency may be as low as 10^{-12} for an airborne system.

II.E. Fluorescent Lifetime Measurements

In an effort to provide a complete study of the various types of algae measurements were made of their fluorescence lifetimes. This was accomplished by observing the pulse width from a phototube having a 2 nsec rise time with a 150 Mhz oscilloscope (2 nsec rise time). The pulse width was found to be 8 ± 2 nsec. This spread in signal is expected since it is the minimum pulse resolution allowed by the detection system. Observation of the fluorescence pulse widths resulted in values of 10 ± 2 nsecs, thereby indicating a 1-2 nsec fluorescence duration.

These values agree well with published data (Brody and Rabinowitch, 1957) which showed that chlorophyll a in solvents has a lifetime of 5.1 - 7.8 nsecs and about 1/4 that value in live cells i.e., 1-2 nsecs.

III. MATHEMATICAL ANALYSIS OF SYSTEM

This section deals with a number of salient environmental and system parameters which must be considered in assessing the feasibility of a remote laser system for the detection and identification of algae. The equations which are given below are used for estimating the signal-to-noise figure for remote detection of the fluorescence signal.

III.A. Calculation of Fluorescence Signal

Figure III-1 is a schematic diagram of a remote laser/detection system being deployed to activate and detect algae. The laser power P_1 incident on the water surface a distance R from the laser/receiver system is given by

$$P_1 = \epsilon_t P_L e^{-\alpha_A^\lambda R} \quad (1)$$

where ϵ_t = transmitter optics efficiency

P_L = primary laser power

α_A^λ = atmospheric attenuation coefficient of the laser

If the reflectivity of the water surface is ρ , the laser power which enters the water, P_2 , is given by equation (2).

$$P_2 = P_1 (1-\rho) \quad (2)$$

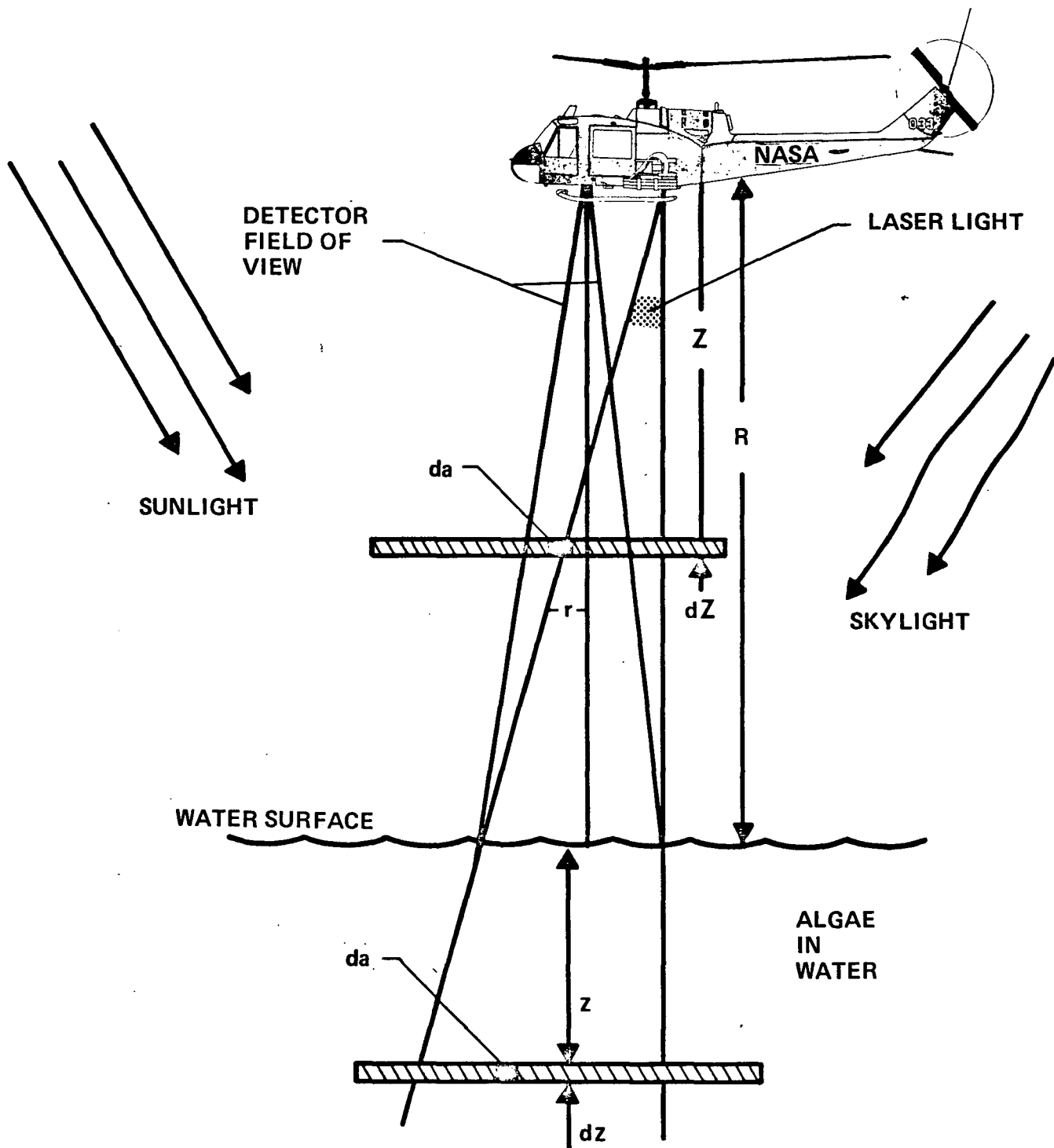


FIGURE III-1
DIAGRAM USED FOR SIGNAL/NOISE CALCULATIONS

The algae may be located at the surface of the water or continue to a depth of \underline{z} below the surface of the water. If the assumption is made that the algae extends uniformly to a depth \underline{h} , the light reaching the algae layer at depth \underline{z} , where $\underline{h} \geq \underline{z}$ is given by

$$P(z) = P_1 (1-\rho) e^{-\alpha_w^\lambda z} \quad (3)$$

where α_w^λ is attenuation coefficient of the water. The power which is absorbed by the water/algae medium in an infinitesimal thickness \underline{dz} is given by

$$\frac{dP(z)}{dz} = -P_1 (1-\rho) e^{-\alpha_w^\lambda z} \alpha_w^\lambda \quad (4)$$

The fluorescence which is generated in this region \underline{dz} can now be calculated via equation (5), i.e.,

$$dP_f = (1-\rho) \alpha_w^\lambda e^{-\alpha_w^\lambda z} \epsilon_{TL} P e^{-\alpha_A^\lambda R} \epsilon_A dz \quad (5)$$

where ϵ_A is the coefficient for producing algal fluorescence within the band of the detector's interference filter. The fluorescence signal which reaches a detector which is co-located with the remote laser transmitter can now be calculated by equation (6)

$$dP_f^D = dP_f (1-\rho) e^{-\alpha_w^{\lambda'} z} e^{-\alpha_A^{\lambda'} R} \frac{A_C \epsilon_C}{4\pi (R+z)^2}$$

$$= (1-\rho)^2 \alpha_w^\lambda e^{-(\alpha_w^\lambda + \alpha_w^{\lambda'})z} \frac{\epsilon_T \epsilon_A \epsilon_C}{4\pi(R+z)^2} e^{-(\alpha_A^\lambda + \alpha_A^{\lambda'})R} \cdot P_L A_C dz \quad (6)$$

where α_w^λ and α_A^λ refer to the absorption of the shifted or fluorescence wavelength in the water/algae medium and the atmosphere respectively, ϵ_L is the collector optics' efficiency and A_C is the area of the receiver. The factor 4π enters the expression since the fluorescence emission is in all directions. Upon integration over all depths, $z \leq h$, it is found that the detected laser induced fluorescence is given by

$$P_{DLAF} = P_L \epsilon_T e^{-(\alpha_A^\lambda + \alpha_A^{\lambda'})R} (1-\rho)^2 \frac{\epsilon_A \epsilon_C A_C}{4\pi R^2} \frac{\alpha_w^\lambda}{\alpha_w^\lambda + \alpha_w^{\lambda'}} \frac{1}{[1 - e^{-(\alpha_w^\lambda + \alpha_w^{\lambda'})h}]} \quad (7)$$

for the case in which $h \ll R$.

III.B. Background Signals

The main sources of background at the detector can be identified by the following notation.

P_{DSAF} = sun generated algal fluorescence at λ'

P_{DKAF} = sky generated algal fluorescence at λ'

P_{DSR} = sun generated reflection at λ'

P_{DKR} = sky generated reflection at λ'

P_{DSAB} = sun generated air backscatter at λ'

P_{DKAB} = sky generated air backscatter at λ'

P_{DSWB} = sun generated water backscatter at λ'

P_{DKWB} = sky generated water backscatter at λ'

Calculation of each of the background components follow.

P_{DSAF} :

As in the derivation of eq. 7, we find that P_{DSAF} = power reaching depth • quantum efficiency • solid angle effect • atmospheric and water attenuation of sun light and fluorescence.

The total irradiance reaching an area element da at depth z is

$$I = \int H_S^\lambda e^{-\alpha_w^{\text{sun}}(\lambda)z} (1-\rho) d\lambda da \quad (8)$$

α_w^{sun} = average attenuation coefficient of sunlight

which generates fluorescent

H_S^λ = the irradiance of the sun

The range of integration is the major absorption region of the algae. The fluorescence which is generated in volume $dzda$ is given by

$$P_f = \int H_S^\lambda e^{-\alpha_w^{\text{sun}}} (\lambda) L (1-\rho) d\lambda \epsilon_A(\lambda) \alpha_w^{\text{sun}} da dz \quad (9)$$

Again as in equation 4 the factor α_w^{sun} represents the proportion of the light reaching z which gets absorbed in a layer dz .

Of the total fluorescence generated

$$G = \epsilon_c (1-\rho) \frac{A_c}{4\pi(R+z)^2} e^{-(\alpha_w^{\lambda'} z + \alpha_A^{\lambda'} R)} \quad (10)$$

arrives at the detector.

Integrating the product of equation (9) and (10) over all depths, $z \leq h$, the absorption bands of the plankton and the area in the view of the detector, results in

$$P_{\text{DSAF}} = \frac{\epsilon_c A_c \Omega_{\text{Fov}} (1-\rho)^2}{4\pi} \epsilon_A H_S^\lambda \alpha_w^{\text{sun}} \frac{e^{-\alpha_A^{\lambda'} R} (1 - e^{-(\alpha_w^{\text{sun}} + \alpha_w^{\lambda'}) h})}{\alpha_w^{\text{sun}} + \alpha_w^{\lambda'}} \quad (11)$$

where H_S^λ is the total solar radiation over the absorption band of the algal, and $R \gg h$.

P_{DKAF} :

As a direct extension of the calculation of P_{DSAF} , P_{DKAF} can be inferred by replacing H_S , the irradiance of the sun with H_K the irradiance of skylight. The results would be

$$P_{DKAF} = \frac{\epsilon_c A_c \Omega_{Fov} (1-\rho)^2}{4\pi} \epsilon_A H_K^\lambda \alpha_w^{\text{sun}} \frac{e^{-\alpha_A^{\lambda'} R} [1 - e^{-(\alpha_w^{\text{sun}} + \alpha_w^{\lambda'}) h}]}{\alpha_w^{\text{sun}} + \alpha_w^{\lambda'}} \quad (12)$$

P_{DSR}:

Total radiation in the field of view is $H_S \Omega_{Fov} R^2$ of which $\rho_w f \frac{A_c \epsilon_c}{2\pi R^2}$ gets to the detector attenuated by $e^{-\alpha_A^{\lambda'} R}$. Here $\rho_w f$ is the total reflected power from the water surface weighted by f for the relative position on the sun and the detector, the sea state, etc., so,

$$P_{DSR} = \frac{H_S^{\lambda'} \Omega_{Fov} A_c \epsilon_c}{2\pi} e^{-\alpha_A^{\lambda'} R} \rho_w f^S \quad (13)$$

Here we have assumed that the reflected light is distributed over 2π steradians.

P_{DKR}:

This result follows directly from the result of the calculation of P_{DSR} . As before we may replace H_S with H_K and get

$$P_{DKR} = \frac{H_K^{\lambda'} \Omega_{Fov} A_c \epsilon_c}{2\pi} e^{-\alpha_A^{\lambda'} R} \rho_w f^K \quad (14)$$

P_{DSAB}:

The power reaching an area element da is $H_S da$. A fraction β_A of this power gets scattered toward the detector, and is attenuated by

$$e^{-\alpha_A^{\lambda'} (z^2 + r^2)^{1/2}}$$

The total detected power is approximately

$$\int_0^R \frac{A_c \epsilon_c}{2\pi} H_S^{\lambda'} \beta_A e^{-\alpha_A^{\lambda'} z} \Omega_{Fov} z^2 dz$$

where we have assumed that $z \gg r$. This is equivalent to stating that the detector has a small field of view.

So,

$$P_{DSAB} = \frac{A_c \epsilon_c}{2\pi} H_S^{\lambda'} \beta_A \frac{(1 - e^{-\alpha_A^{\lambda'} R})}{\alpha_A^{\lambda'}} \Omega_{Fov} \quad (15)$$

P_{DKAB} :

Again, we may infer the skylight result from P_{DSAB} by replacing H_S with H_K . Thus,

$$P_{DKAB} = \frac{A_c \epsilon_c}{2\pi} H_K^{\lambda'} \beta_A \frac{(1 - e^{-\alpha_A^{\lambda'} R})}{\alpha_A^{\lambda'}} \Omega_{Fov} \quad (16)$$

where β_A is the air backscatter coefficient for the wavelength transmitted by the interference filter on the detector.

P_{DSWB} :

This calculation may be performed by adapting the approach of the calculation of P_{DSAB} . The power reaching an area da in the water is

$$H_S^{\lambda'} e^{-\alpha_w^{\lambda'} z} (1 - \rho) da$$

Of this β_w gets scattered in a layer dz toward the surface of which $(1 - \rho) e^{-\alpha_w^{\text{sun}} z}$ gets through. Thus, the total backscattered

light is

$$H_S^{\lambda'} e^{-2\alpha_W^{\lambda'} z} (1-\rho)^2 \beta_W^{\lambda'} dz e^{-\alpha_A^{\lambda'} R} da$$

Thus the total detected signal generated in the field of view of the detector which reaches the detector is

$$\begin{aligned} & \int H_S^{\lambda'} e^{-2\alpha_W^{\lambda'} z} (1-\rho)^2 \beta_W^{\lambda'} e^{-\alpha_A^{\lambda'} R} \frac{A_c \epsilon_c}{2\pi(R+z)^2} da dz \\ &= \int_0^\infty H_S^{\lambda'} e^{-2\alpha_W^{\lambda'} z} (1-\rho)^2 \beta_W^{\lambda'} e^{-\alpha_A^{\lambda'} R} \frac{A_c \epsilon_c}{2\pi} \Omega_{Fov} dz \\ P_{DSWB} &= H_S^{\lambda'} (1-\rho)^2 \beta_W^{\lambda'} e^{-\alpha_A^{\lambda'} R} \frac{A_c \epsilon_c \Omega_{Fov}}{4\pi\alpha_W^{\lambda'}} \end{aligned} \quad (17)$$

P_{DKWB}:

Using the result obtained above and substituting H_K for H_S one finds the skylight generated water backscatter signal to be

$$P_{DKWB} = H_K^{\lambda'} (1-\rho)^2 \beta_W^{\lambda'} e^{-\alpha_A^{\lambda'} R} \frac{A_c \epsilon_c \Omega_{Fov}}{4\pi\alpha_W^{\lambda'}} \quad (18)$$

All of the background signals taken together form, P_B , the background noise.

III.C. Analysis of Radiative Transfer Equations

Of some importance is the change in P_{DLAF} and signal to noise ratio as a function of altitude. If we

rewrite the appropriate equations in the following way,
we get the dependence of the various signals on R.

First P_{DLAF} goes as

$$\frac{e^{-(\alpha_A^\lambda + \alpha_A^{\lambda'})R}}{R^2}$$

for simplicity we may write it as

$$P_{DLAF} = \frac{\alpha}{R^2} e^{-(\alpha_A^\lambda + \alpha_A^{\lambda'})R}$$

where

$$\alpha = P_L \epsilon_T \frac{(1-\rho)^2 \epsilon_A \epsilon_C^A}{4\pi} \frac{\alpha_w^\lambda}{\alpha_w^\lambda + \alpha_w^{\lambda'}} [1 - e^{-(\alpha_w^\lambda + \alpha_w^{\lambda'})h}] \quad (19)$$

which depends on λ since it includes α_w^λ .

Other terms include:

$$P_{DSAF} = \beta e^{-\alpha_A^{\lambda'} R} \text{ and } P_{DKAF} = \beta' e^{-\alpha_A^{\lambda'} R}$$

where

$$\beta = \frac{\epsilon_C^A \Omega_{Fov} (1-\rho)^2}{4\pi} \frac{\alpha_w^{\text{sun}} \epsilon_A^{H_S}}{\alpha_w^{\text{sun}} + \alpha_w^{\lambda'}} [1 - e^{-(\alpha_w^{\lambda} + \alpha_w^{\lambda'})h}] \quad (20)$$

and

$$\beta' = \frac{\epsilon_C^A \Omega_{Fov} (1-\rho)^2}{4\pi} \frac{\alpha_w^{\text{sky}} \epsilon_A^{H_K}}{\alpha_w^{\text{sky}} + \alpha_w^{\lambda'}} [1 - e^{-(\alpha_w^{\lambda} + \alpha_w^{\lambda'})h}] \quad (21)$$

$$P_{DSR} = \phi e^{-\alpha_A^{\lambda'} R} \text{ and } P_{DKR} = \phi' e^{-\alpha_A^{\lambda'} R}$$

with

$$\phi = H_S^{\lambda'} \frac{\Omega_{Fov} A_C \epsilon_C \rho_w f_w^S}{2\pi} \quad (22)$$

and

$$\phi' = \frac{H_K^{\lambda'} \Omega_{Fov} A_C \epsilon_C \rho_w f_w^K}{2\pi} \quad (23)$$

$$P_{DSAB} = \delta (1 - e^{-\alpha_A^{\lambda'} R}) \quad \text{and} \quad P_{DKAB} = \delta' (1 - e^{-\alpha_A^{\lambda'} R})$$

with

$$\delta = \frac{A_C \epsilon_C}{2\pi} \frac{H_S^{\lambda'} \beta_A^{\lambda'}}{\alpha_A^{\lambda'}} \Omega_{Fov} \quad (24)$$

and

$$\delta' = \frac{A_C \epsilon_C}{2\pi} \frac{H_K^{\lambda'} \beta_A^{\lambda'}}{\alpha_A^{\lambda'}} \Omega_{Fov} \quad (25)$$

$$P_{DSWB} = \psi e^{-\alpha_A^{\lambda'} R} \quad \text{and} \quad P_{DKWB} = \psi' e^{-\alpha_A^{\lambda'} R}$$

with

$$\psi = H_S^{\lambda'} (1-\rho)^2 \beta_w^{\lambda'} \frac{A_C \epsilon_C \Omega_{Fov}}{4\pi \alpha_w^{\lambda'}} \quad (26)$$

and

$$\psi' = H_K^{\lambda'} (1-\rho)^2 \frac{\beta_w^{\lambda'} A_C \epsilon_C \Omega_{Fov}}{4\pi \alpha_w^{\lambda'}} \quad (27)$$

TABLE III
DESCRIPTION

SYMBOL	DESCRIPTION	VALUE	REFERENCE
ϵ_L	transmitter optics efficiency	.5	Estimate
ϵ_C	receiver optics efficiency	.1	Estimate
α_A^{337}	atmosphere attenuation of 337 nm radiation	.24km ⁻¹	McClatchey et al
α_A^{600}	atmosphere attenuation of 600 nm radiation	.064 km ⁻¹	Taylor & Yates
$\alpha_A^{\lambda'}$	atmosphere attenuation of 685 nm radiation	.064km ⁻¹	Taylor & Yates
$H_S^{\lambda'}$	Solar irradiance (10nm band at 685)	14.5x10 ⁶ $\frac{W}{km^2}$	Jensen
$H_K^{\lambda'}$	sky irradiance (10nm band at 685)	7x10 ⁶ $\frac{W}{km^2}$	Estimate
Ω_L	solid angle of laser beam	4x10 ⁻⁶ Sr	Estimate
Ω_E	solid angle of emission	$\pi, 2\pi, 4\pi$	Estimate
Ω_{Fov}	solid angle receiver	4x10 ⁻⁶ Sr	Estimate
A_C	collecting optic size	7.2x10 ⁻⁸ Km ²	Estimate
A_T	area of target ($\Omega_L R^2$)	3.6x10 ⁻⁷ Km ²	Estimate
R	altitude of aircraft	.2Km	Estimate
ρ	reflectance of water	.05	Sverdrup et al
ϵ_A	quantum efficiency of algae	10 ⁻³	Measured
H_S	sun power in band from 300 to 400 nm	10 ⁸ W/Km ²	Koller

continued.....

SYMBOL	DESCRIPTION	VALUE	REFERENCE
f_w^S	sunlight reflectance function	10^{-3}	Fantasia
f_w^K	skylight reflectance function	1	Fantasia
$+a_w^{337}$	water attenuation at 337	240 Km^{-1}	Measured
$+a_w^{600}$	water attenuation at 600	670 Km^{-1}	Measured
$+a_w^{\lambda'}$	water attenuation at 685	1600 Km^{-1}	Measured
$\beta_A^{\lambda'}$	atmospheric backscatter	$9.43 \times 10^{-3} \text{ Km}^{-1}$	Estimate*
H_K	skylight power in band 300 to 400 nm	$.5 \times 10^8 \text{ w/Km}^2$	Estimate
$\beta_w^{\lambda'}$	water backscatter	$.8 \text{ Km}^{-1}$	Estimate*
η	detector quantum efficiency	.12	Manufacturers data
B	post detection bandwidth	150 Mhz	Estimate
h	Planck's constant	$6.6 \times 10^{-27} \text{ erg-sec}$	
P_L	laser power		
λ'	fluorescence wavelength	685 nm	Measured
α_w^{sun}	absorption of 300 to 400 nm band	850 Km^{-1}	Estimate

+These values are representative of the value found in natural water. Measurements are represented in Section IIB.

*These data have been estimated from values given for other wavelengths by Fantasia et al.

The signal to noise power ratio, S/N is given by Ross (1966) is

$$S/N = \eta [P_{DLAF}]^2 / 2Bh\nu P_B \quad (28)$$

where η is the quantum efficiency of the photodetector

B is the post detection bandwidth

$h\nu$ is the energy per photon

Using the previous calculations, S/N may be rewritten as

$$\frac{S}{N} = \frac{\eta \frac{\alpha^2}{R^4}}{2Bh\nu} \frac{e^{-2(\alpha_A^\lambda + \alpha_A^{\lambda'})R}}{[\beta + \beta' + \phi + \phi' - \delta - \delta' + \psi + \psi'] e^{-\alpha_A^{\lambda'} R} + \delta + \delta'} \quad (29)$$

This may be put in a more workable form by replacing the greek letters with their numerical values. They are as follows (from values given in Table III).

$\phi = .332 \times 10^{-11} w$	$\phi' = .166 \times 10^{-8} w$
$\delta = 7.83 \times 10^{-9} w$	$\delta' = 3.9 \times 10^{-9} w$
$\psi = 1.5 \times 10^{-11} w$	$\psi' = .75 \times 10^{-11} w$
$\beta = .45 \times 10^{-11} w$	$\beta' = .22 \times 10^{-11} w$
$\alpha = 7 \times 10^{-9} wKm^2$ for a 600 nm laser	
$= 3 \times 10^{-9} wKm^2$ for a 337 nm laser	

Here we have used the values presented earlier as well as assumed that 10% of the fluorescence is passed by the interference filter on the detector and that the q value

for algae is 1%. Thus $\epsilon_A \approx 10^{-3}$. Clearly the value of α depends on the laser wavelength used. For this reason both the 600 nm and 337 nm cases will be calculated assuming a 100 kw laser.

For a 600 nm laser

$$\frac{S}{N} = \frac{68}{R^4} \frac{e^{-.192R}}{11.73 - 10.13 e^{-.064R}} \quad (30)$$

For a 337 nm laser

$$\frac{S}{N} = \frac{12.5}{R^4} \frac{e^{-.544R}}{11.73 - 10.13 e^{-.064R}}$$

The signal is expressed as

600 nm

$$S = \frac{7 \times 10^{-9}}{R^2} e^{-(1.28)R} \quad (31)$$

337 nm

$$S = \frac{3 \times 10^{-9}}{R^2} e^{-(.304)R}$$

It should be noted that the signal to noise ratio derived above is a power ratio, not a voltage ratio as is normally measured. A simple relationship exists between the power ratio and the voltage ratio, however, since power is proportional to the square of the detected current while voltage is proportional to the current. Thus

$$\left(\frac{S}{N} \right)_{\text{power}} = \left[\left(\frac{S}{N} \right)_{\text{voltage}} \right]^2 \quad (32)$$

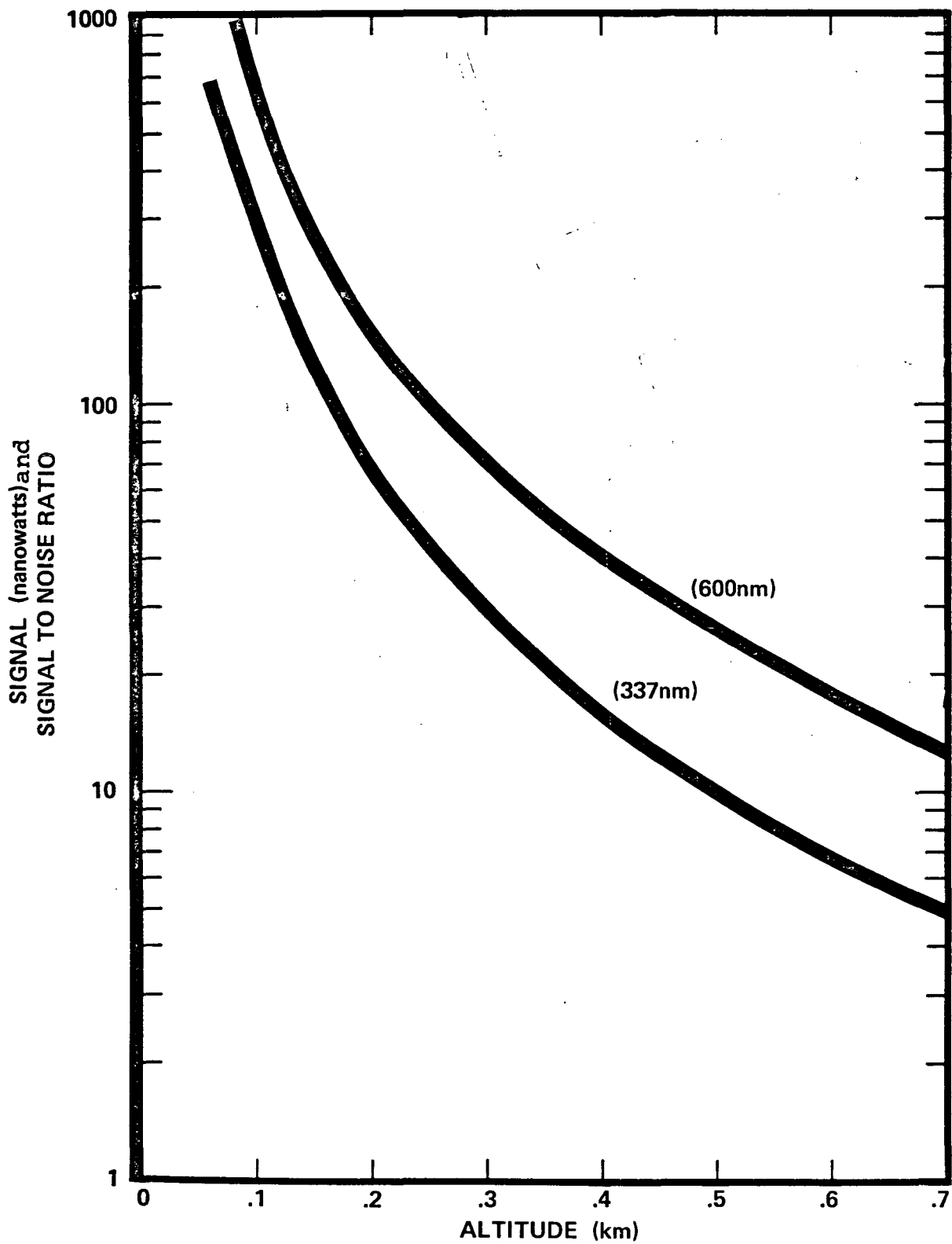


FIGURE III-2

Figure III-2 shows the plot of both the signal/noise (equation 32) and the signal (equation 31) for excitation at 337 and 600 nm. The superiority of using the longer wavelength as the source of excitation of the algae is clearly demonstrated in Fig. III-2. Not only does this wavelength (600 nm) provide a sizable advantage in the detected signal level, but also results in a marked increase in the signal-to-noise ratio over that obtained from excitation at 337 nm.

Although excitation at 600 nm has distinct advantages, as previously shown, over that at 337 nm, it should be noted that detection of algal fluorescence at 685 nm should be possible using a 100 kw peak pulse laser at either wavelength from an altitude as great as 0.5 km. The minimum concentration of chlorophyll which could be detected in this case is estimated to be 1.0 mg/m^3 .

The reader should note that the fact that the signal and signal-to-noise voltage ratio overlap for each of the wavelengths of interest is mere coincidence and should not be construed to represent a general result.

IV. CONCLUSIONS AND RECOMMENDATIONS

Results of the literature search which are described in Section I and Appendix A show that a fair amount of information is available regarding algae which are normally found in the Chesapeake Bay. In addition, information is also available on the photosynthetic pigments which make up the character of the various classes of the Bay algae. However, the search revealed that information was incomplete on the absorption, excitation and fluorescence spectra for many of these algal species. Little to no information was available for the optical attenuation coefficients and quantum efficiencies of these algae. Lastly, no measurements except those reported by Hickman and Moore (1970) were available for laser excitation of algae.

The results of the experiments described in Section II are summarized below:

1. Each of the algal classes examined have unique pigment components although not all of these pigments fluoresce.
2. All of the classes contain at least some chlorophyll a.
3. The quantum efficiencies which were measured for the various algal species are roughly the same; i.e., varying from 0.2 to 3 percent.
4. The absorption spectra for the algae were peaked at approximately 400 nm, dipped at about 500 nm, and again peaked in

the 600-700 region.

5. The fluorescence spectra varied considerably from specie to specie.

On the basis of these data, it is clear that if one is concerned with detection of chlorophyll a only, excitation could take place at either absorption maxima; i.e., 400 ± 50 or 600 ± 50 nm, while detection is made at 685 nm. However, if this fluorescence technique is to be used for specie identification, excitation and detection must be made at more than the wavelength.

The development of a specie identification system would present substantially more problems than those encountered for a chlorophyll a detection system. However, a well-designed system should be able to provide complete profiles of the individual classes of algae in water.

From signal/noise calculation it appears feasible to measure chlorophyll a in concentrations as low as 1.0 mg/m^3 using a laser operating at 100 kw/peak pulse power from an altitude of 500 meters. Although excitation of chlorophyll a fluorescence can be made at either the 400 or 600 nm region there appear to be substantial advantages (signal; signal/noise considerations) for exciting the chlorophyll a fluorescence at the longer wavelength.

Future Research

In order to advance the technique of using laser stimulated fluorescence of algae to the point where a feasible system can

be developed for the purpose of identifying and mapping algae the following areas of research should be pursued.

1. Perform measurements of the absorption, excitation and fluorescence spectra for the remaining algal species which are of interest to the marine environment. In situ measurements should be made on those algal species which are extremely fragile and cannot be transported to the laboratory.

2. Investigation of various environmental parameters which effect the fluorescence and stability of the various algal species. These will include the affects of (a) temperature (b) salinity (c) ambient light (d) pH.

3. Additional engineering design on an optimal laser/receiver system in order to maximize detectability of various algal pigments. This will be accomplished by maximizing the signal using improved detectors, and reducing the noise by appropriate filtering and signal processing.

APPENDIX A - BIOLOGICAL SURVEY OF CHESAPEAKE BAY

1. Introduction

It should be emphasized that information on the growth, concentration, yearly and daily cycles and blooms of algae is of utmost importance in determining the optimum system parameters for a remote laser/fluorescence system for detecting and identifying algae. However, the results of the various studies, a summary of which is given in this report, should be used only as a guide of what to expect. It should be noted that there are substantial yearly variations in the various parameters affecting the growth of algae. This review is only intended to suggest probable constituents in the algal population and general features of their distribution in the bay. In order to obtain absolute measurements of algal types or concentration by deployment of a remote laser sensing system it is apparent that discrete ground truth data be obtained simultaneously with the remote data.

A review of the literature reveals that a very limited number of studies have been made on the concentration, type, and distribution of phytoplankton in the Chesapeake Bay. The first comprehensive study of the hydrographics and biology of the bay was undertaken by the U. S. Bureau of Fisheries in 1915-1916 and 1920-21. The data that was collected on

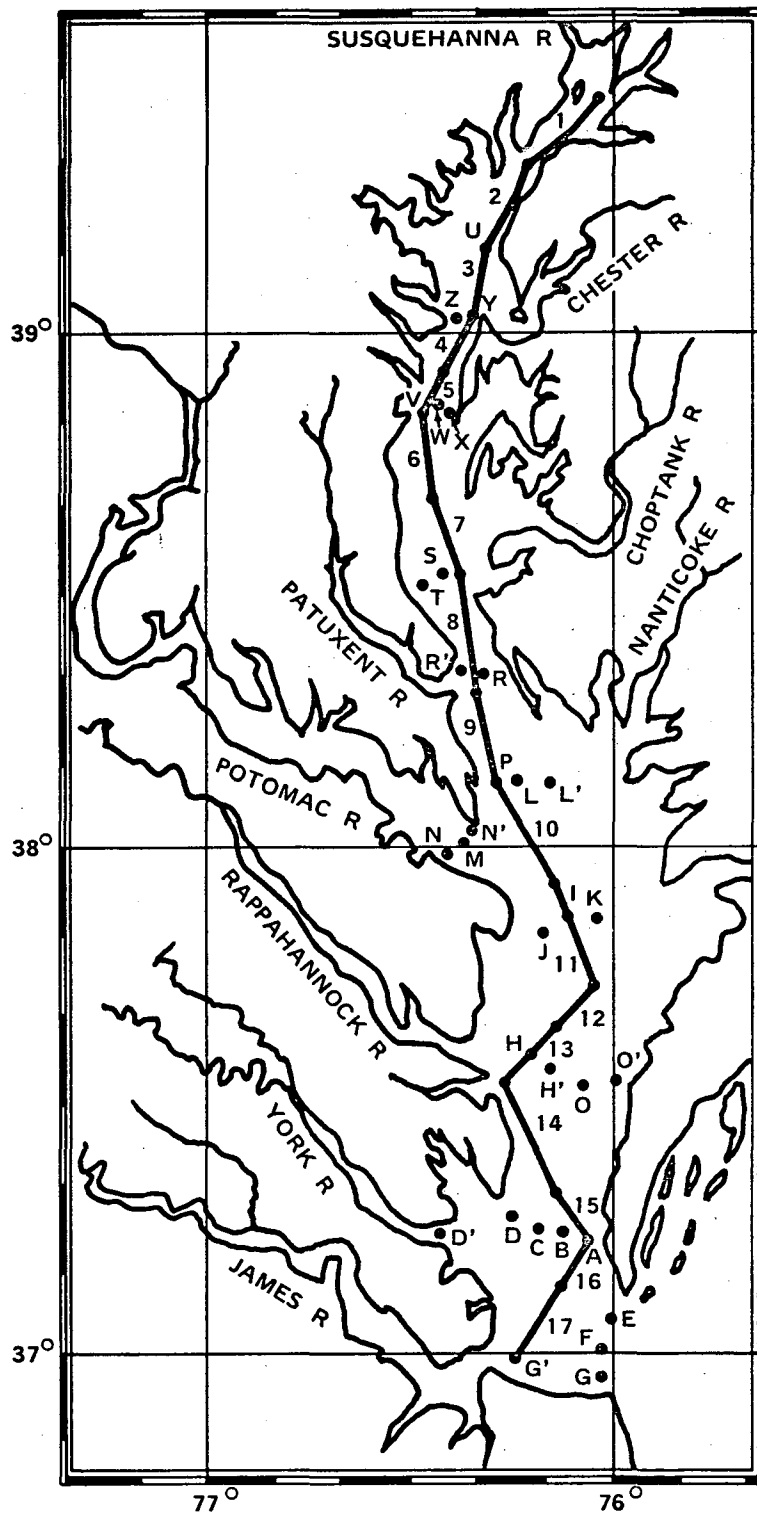
plankton during this study was reported by Wolfe, 1926. A much more quantitative study of the Bay was reported (Whaley and Taylor, 1968). Patten et al, 1963, reported results on various species and concentration of plankton in the lower Bay from the York River mouth to Cape Charles.

These studies have determined the major plankton constituents in the Bay at various times of the year. In general the diatoms (Bacillariophyta) and dinoflagellates (Euglenophyta) dominate the total phytoplankton population of the Bay. There are of course other classes that are also represented although only in modest numbers. Bogorad (1970) gives the result that the dinoflagellates have chlorophyll a and b, β -carotene and other carotenes as yet unidentified. On the other hand, the diatoms have chlorophylls a and c and β -carotene and ϵ -carotene. In some species only the α -carotenes are seen to exist.

2. Distribution of Algae

As previously mentioned, the work reported by Wolfe et al (1926) was the first comprehensive study of algae in Chesapeake Bay. Figure A-1 shows the stations where the measurements were made (indicated by A-W)*. Measurements of the average number of diatoms were recorded at each station. In addition in order to measure the diurnal variation of the concentration, station U was monitored for a 24 hour period. The vertical distribution of the diatoms was also measured,

*These sites also include those indicated by primes.



CHESAPEAKE BAY CRUISE
COURSE FOR PLANKTON SURVEYS

FIGURE A-1

along with a measurement of salinity and temperature. It has been pointed out (Patten et al, 1963) that these samples were taken by a centrifuge method which may have destroyed the flagellate populations which make up a large constituent in the population. This report has been assessed to provide only qualitative information of the plankton in the Bay.

A much more careful study of the lower Bay was provided by Patten et al, 1963. Figure A-1 shows the position of the five measurement stations. (A, B, C, D. and E.) At each station water samples were taken at both the surface and the bottom. Temperature, dissolved oxygen, nutrients, chlorophyll and seston were measured for each sample. Chlorinity varied from 9.9‰ to 13.7‰ for surface water and 11 to 14.6‰ at the bottom. Dissolved oxygen was found in levels of 8-9 mg/l. The turbidity, as defined by an optical attenuation coefficient α , was found to vary from 0.5m^{-1} at the surface to $0.5 - 2.5\text{m}^{-1}$ near the bottom.

The most complete study done to date on the plankton of the Bay has been done by Whaley and Taylor (1968). Continuous monitoring was made of salinity, temperature, total diatoms, total dinoflagellates and numbers of various individual species from the head of the Bay to the mouth. Figure A-1 shows the path (indicated by the numbers) used

by the research vessel while making these measurements, while Figs. A-2 and A-3 show the temperature and salinity profiles made during this study respectively. The salinity distribution reflects the seasonal changes in the flow rates of the river feeding the Bay.

Figure A-4 is a graph of the total monthly concentration of diatoms that was observed in the Bay while Figs. A-5 through A-10 give the concentrations for the specific diatoms.

Fig. A-5 *Cyclotella* sp profile

Fig. A-6 *Chaetoceros* sp profile

Fig. A-7 *Rhizosolenia fragilissima* profile

Fig. A-8 *Nitzschia pungens* Var *Atlantica* profile

Fig. A-9 *Skeletonema costatum* profile

Fig. A-10 *Thalassionema nitzschioides* profile

Fig. A-11 shows the monthly concentration of the total dinoflagellates, while Figs. A-12 through A-15 show the monthly variation in the location and concentration of the specific dinoflagellates.

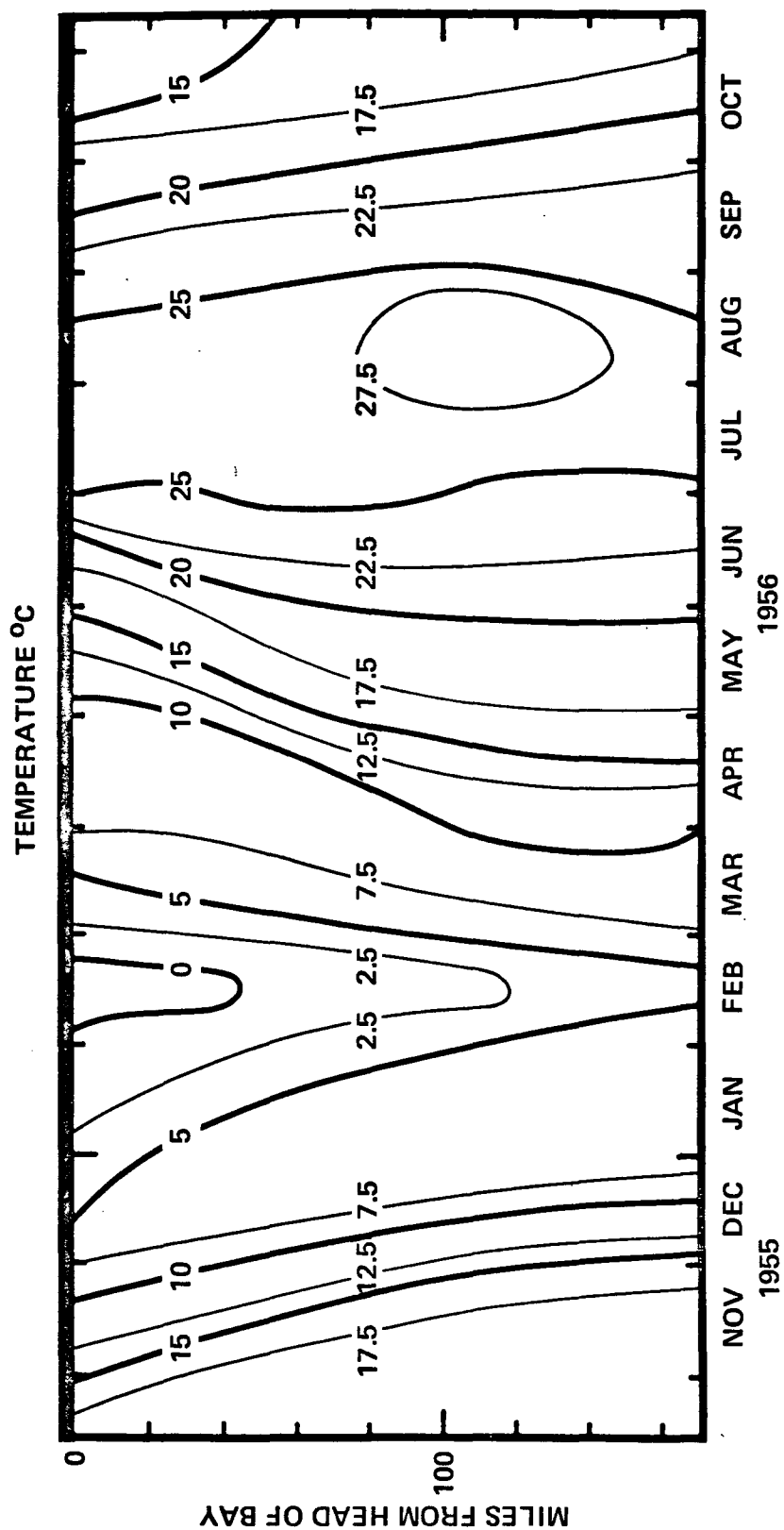
Fig. A-12 *Prorocentrum micans* profile

Fig. A-13 *Exuviella* sp profile

Fig. A-14 *Peridinium leonis* profile

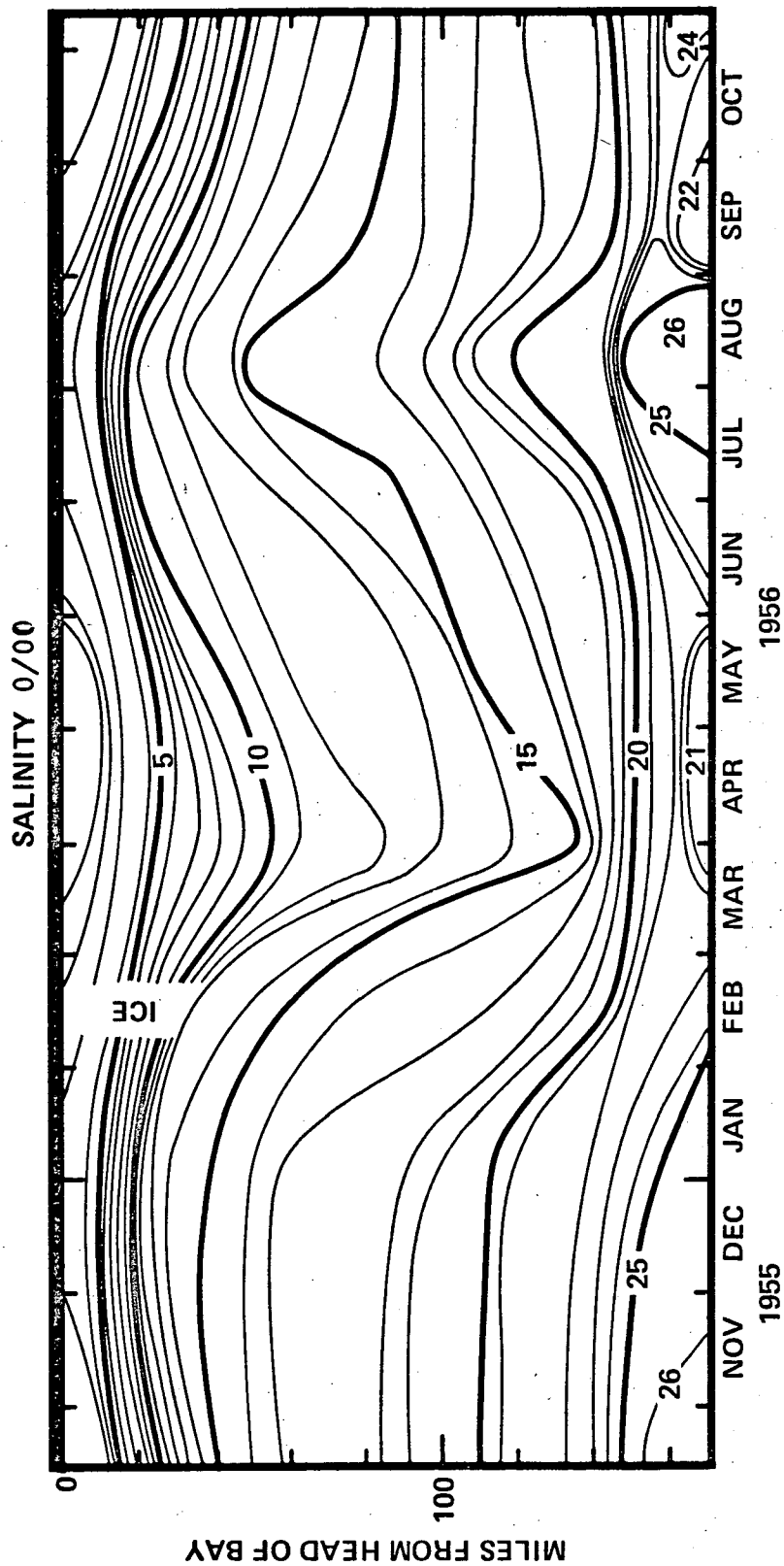
Fig. A-15 *Ceratium furca* profile

From December through February light intensities are low, water temperatures are low and there is substantial



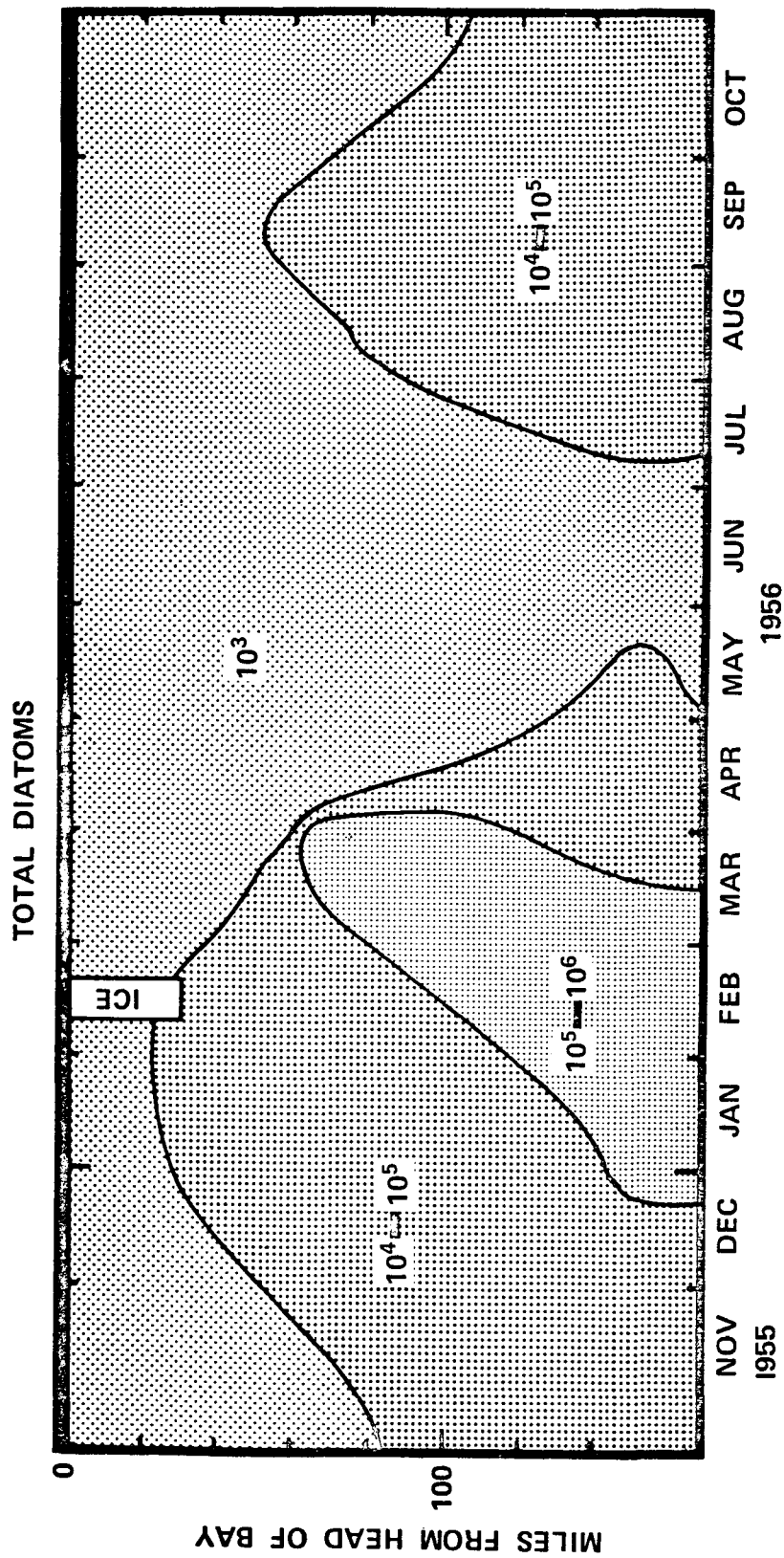
TEMPERATURE PROFILE FOR CHESAPEAKE BAY. THIS AND ALL OTHER PROFILES ARE FOUND IN WHALEY AND TAYLOR, 1968

FIGURE A-2



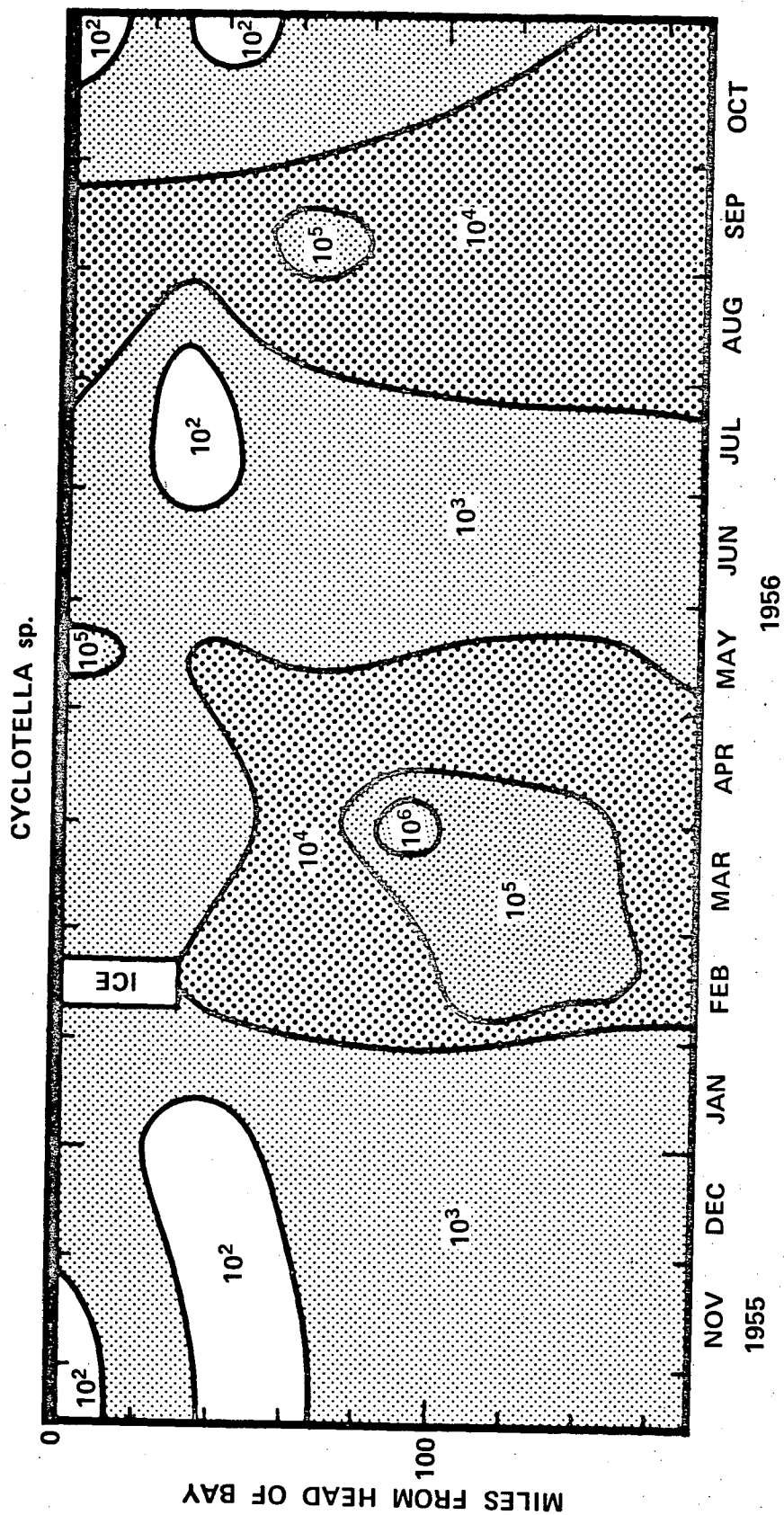
SALINITY DISTRIBUTION IN CHESAPEAKE BAY

FIGURE A-3



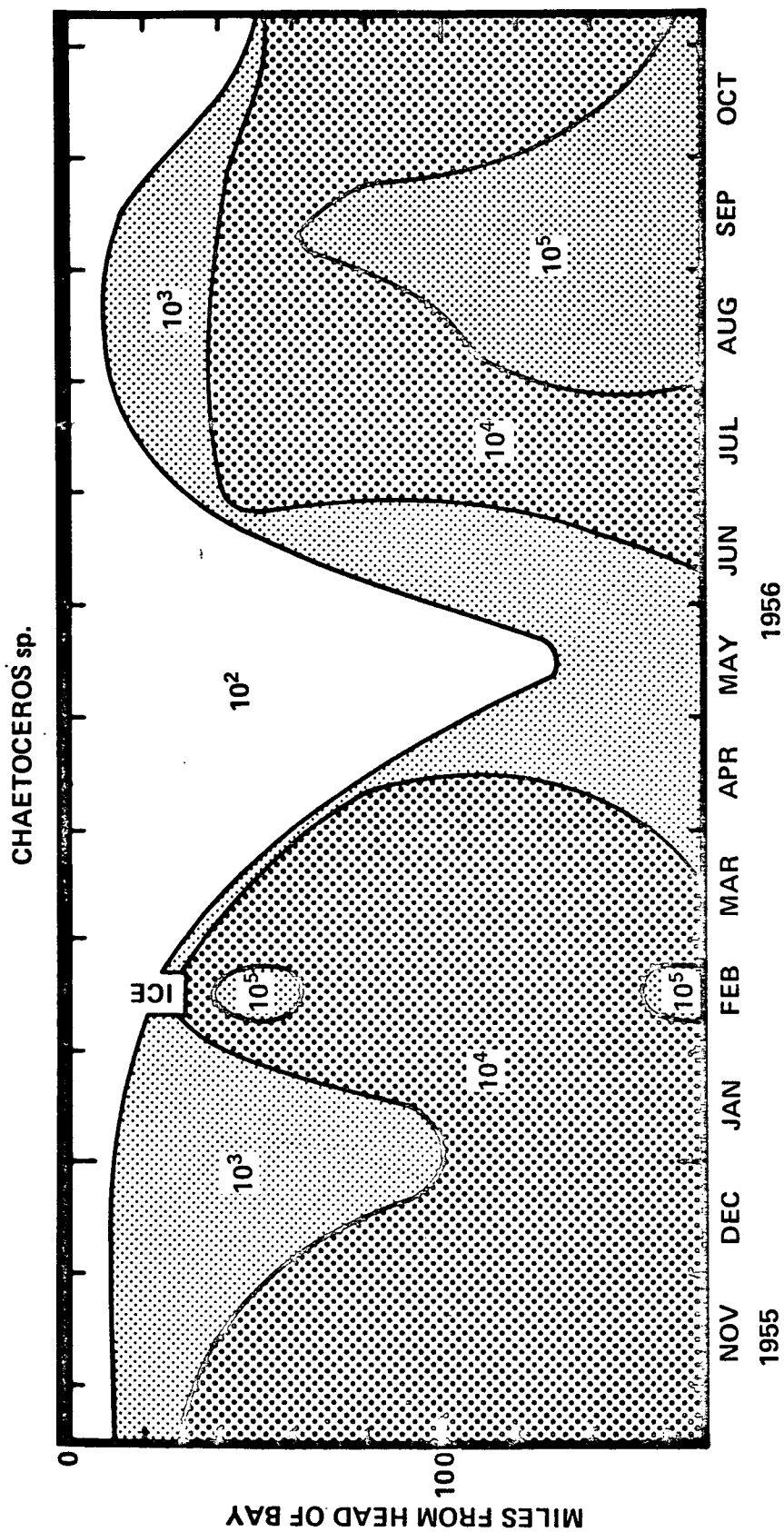
DIATOM PROFILE. NUMBERS IN THIS AND OTHER PROFILES INDICATE THE NUMBER OF CELLS PER LITER.

FIGURE A-4



CYCLOTELLA sp. PROFILE

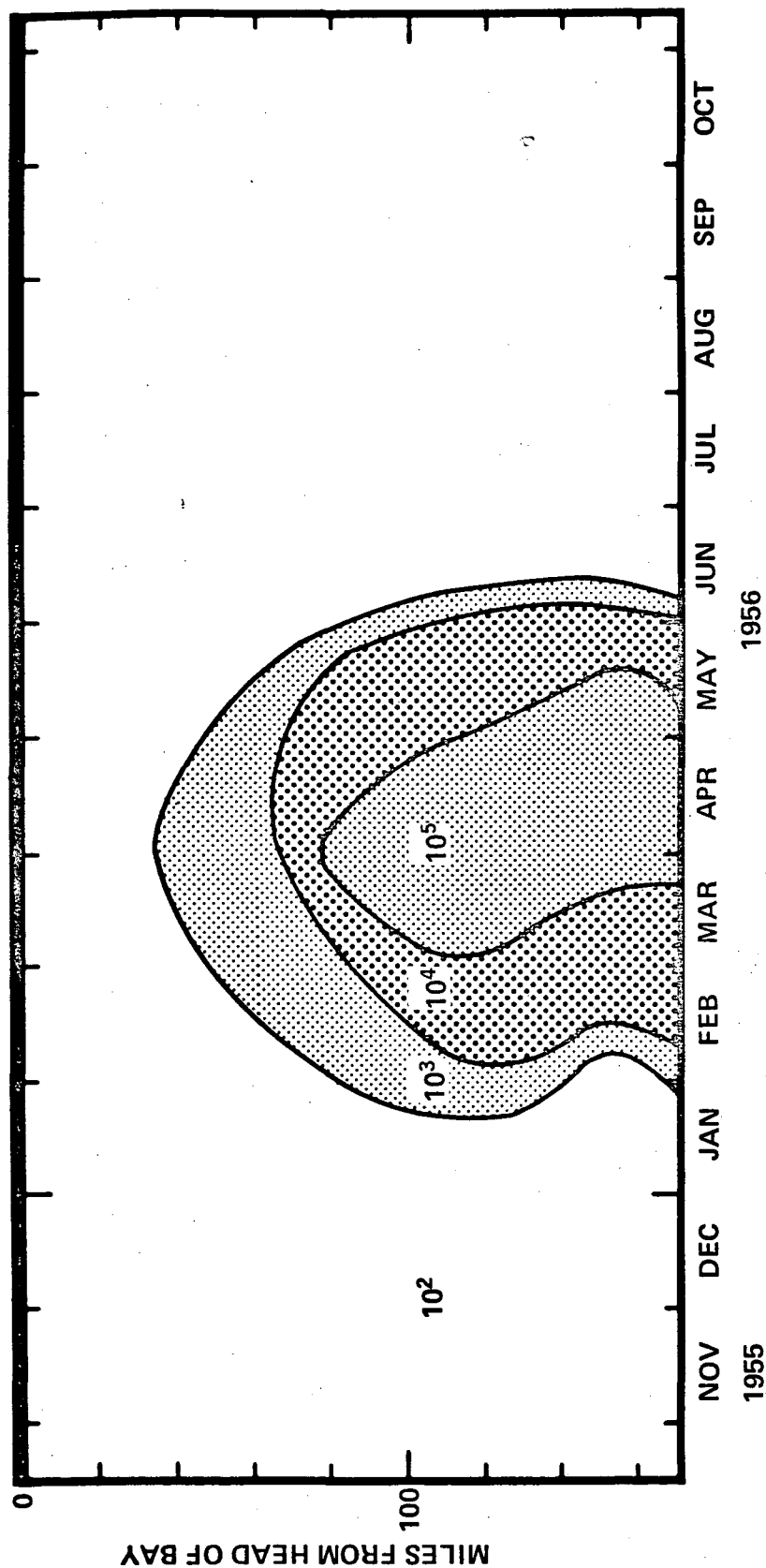
FIGURE A-5



CHAETOCEROS sp. PROFILE

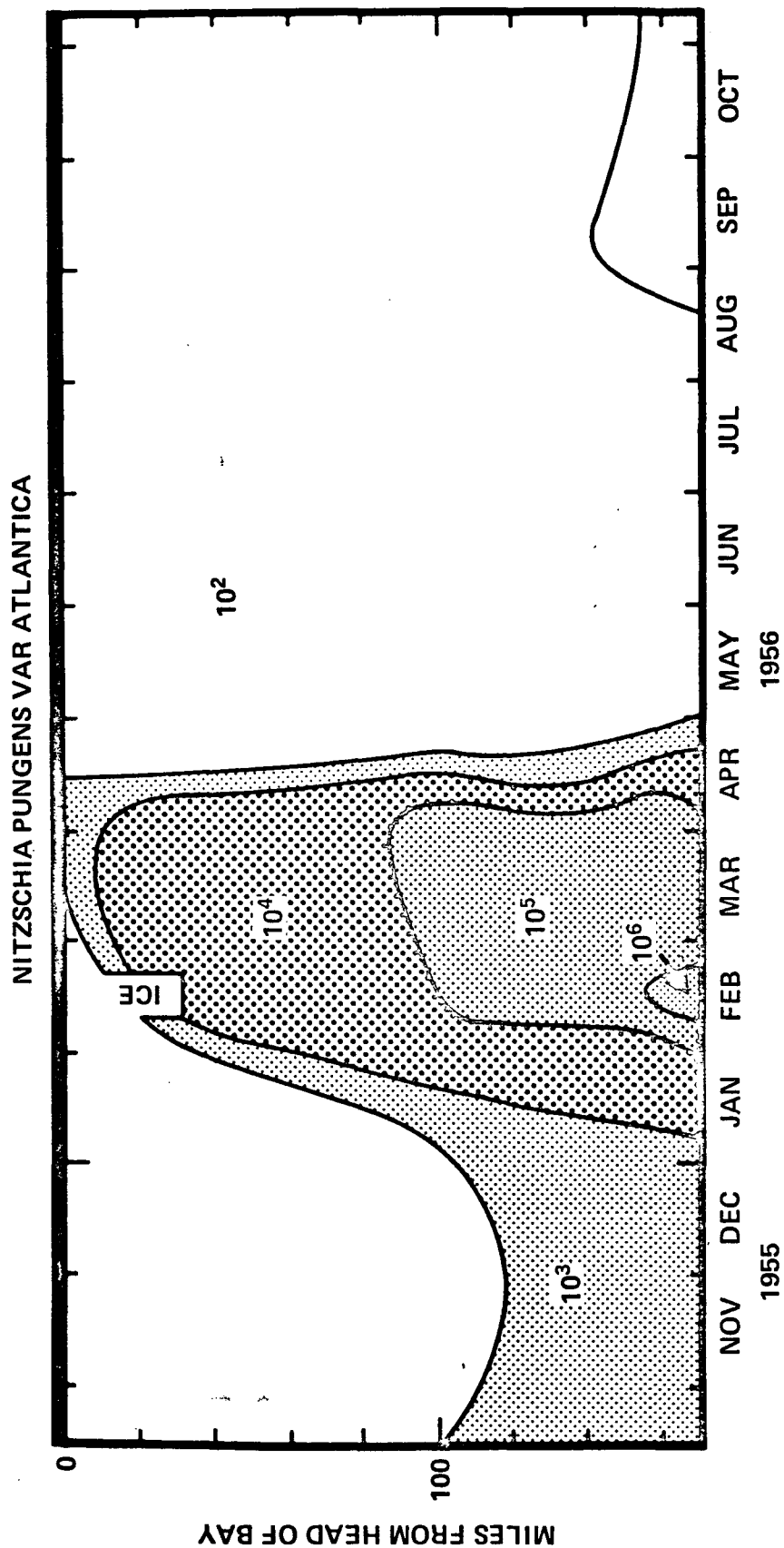
FIGURE A-6

RHIZOLENIA FRAGILISSIMA



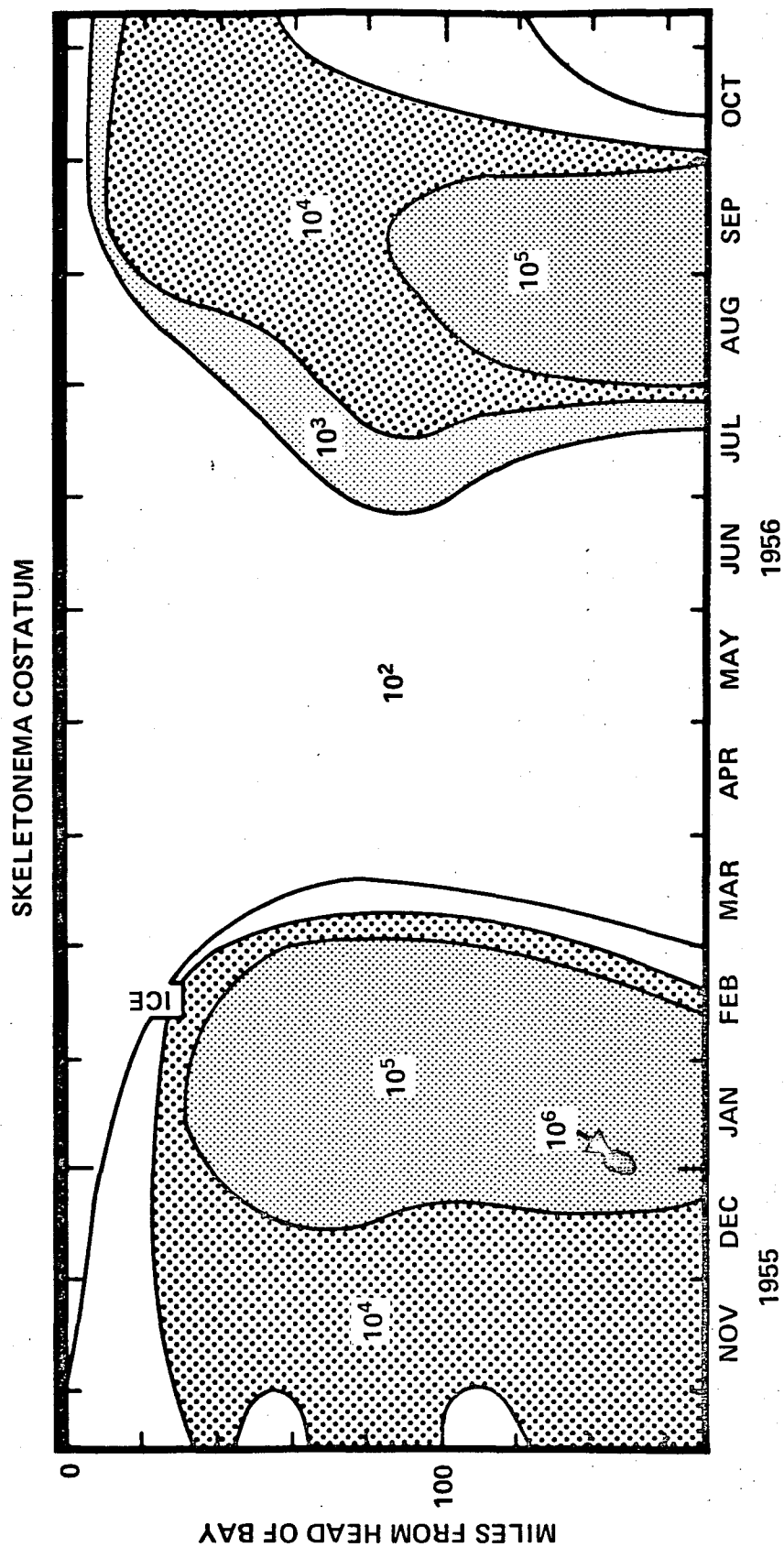
RHIZOLENIA FRAGILISSIMA PROFILE

FIGURE A-7



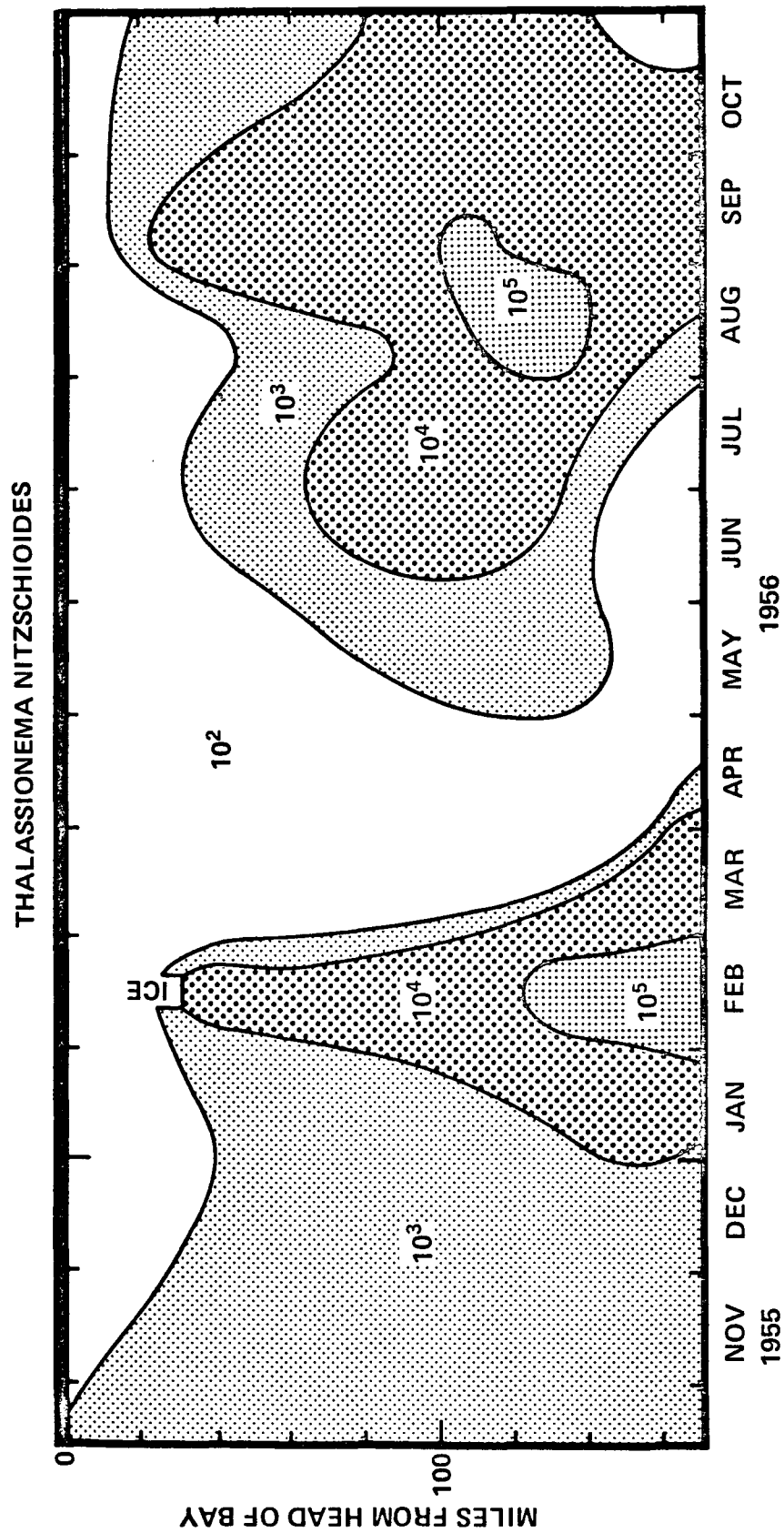
NITZSCHIA PUNGENS VAR ATLANTICA PROFILE

FIGURE A-8



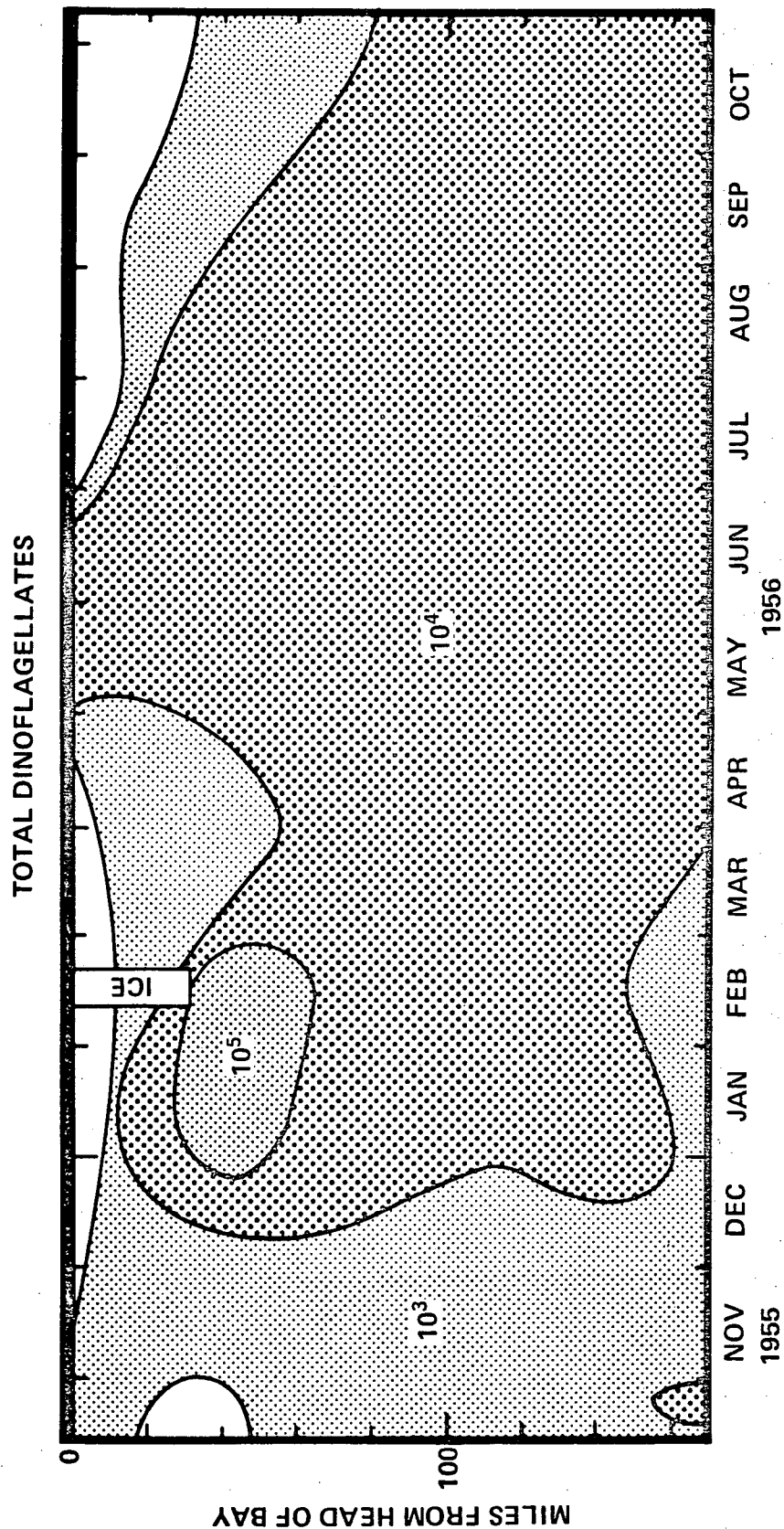
SKELETONEMA COSTATUM PROFILE

FIGURE A-9



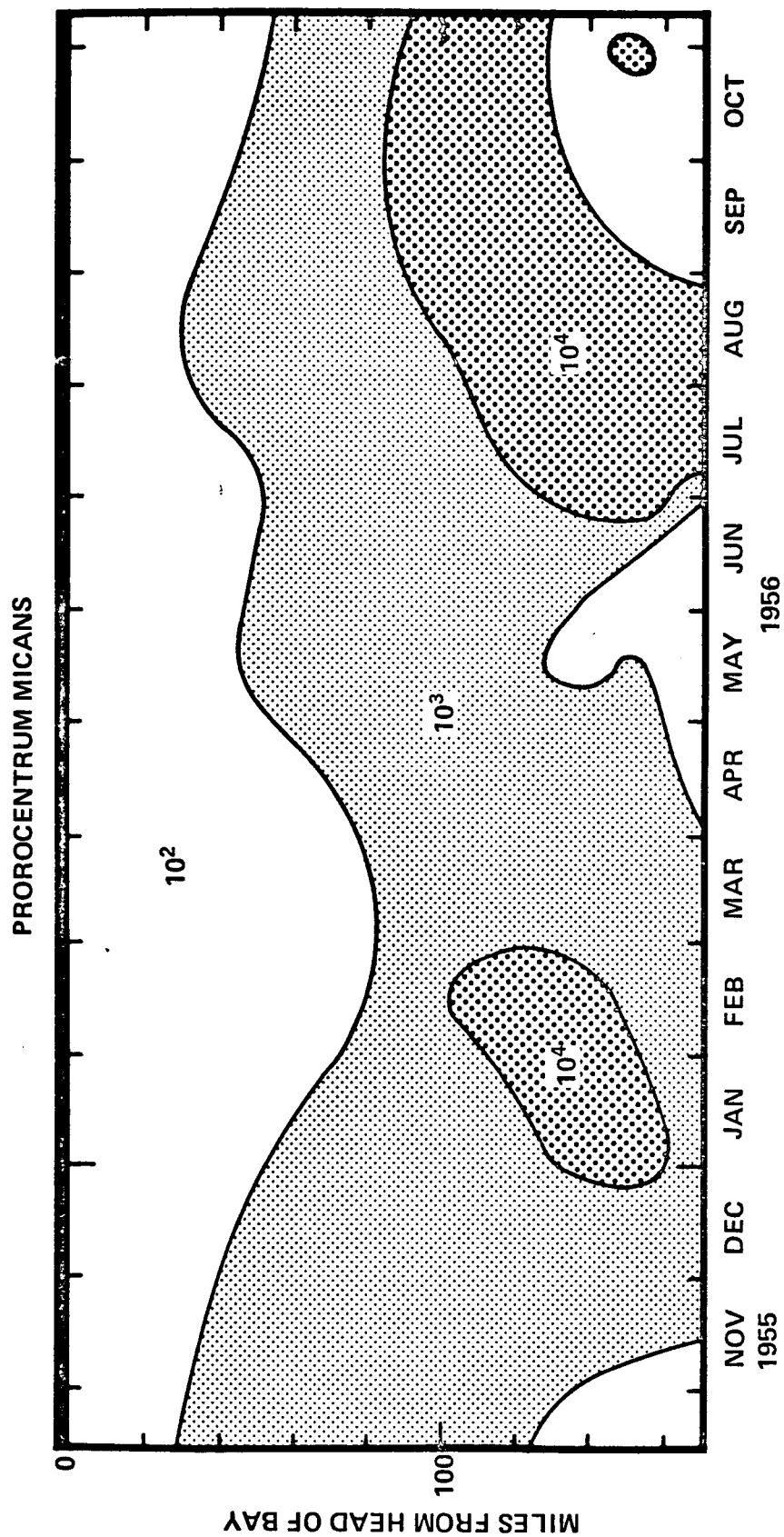
THALASSIONEMA NITZSCHIOIDES PROFILE

FIGURE A-10



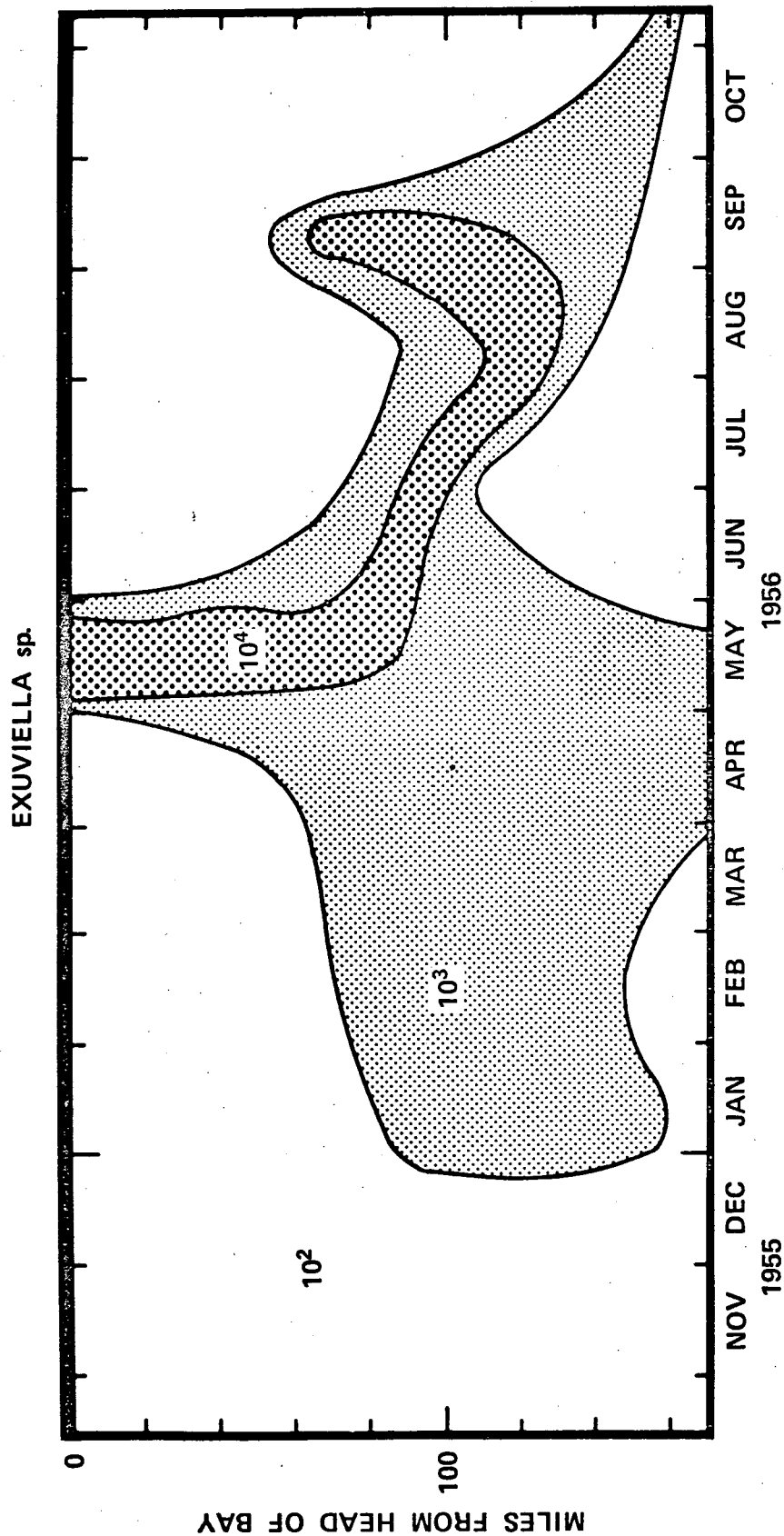
TOTAL DINOFLAGELLATES PROFILE

FIGURE A-11



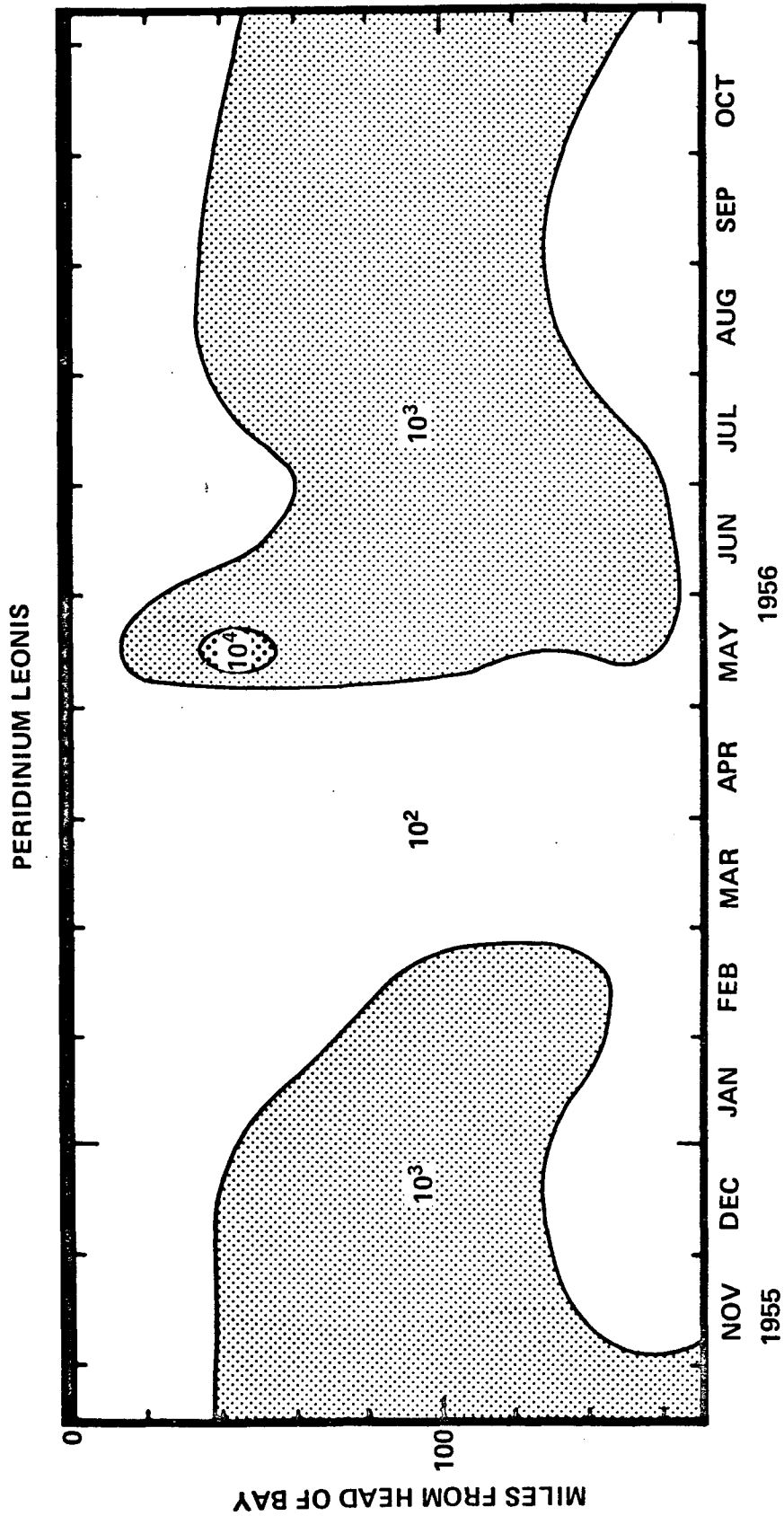
PROROCENTRUM MICANS PROFILE

FIGURE A-12



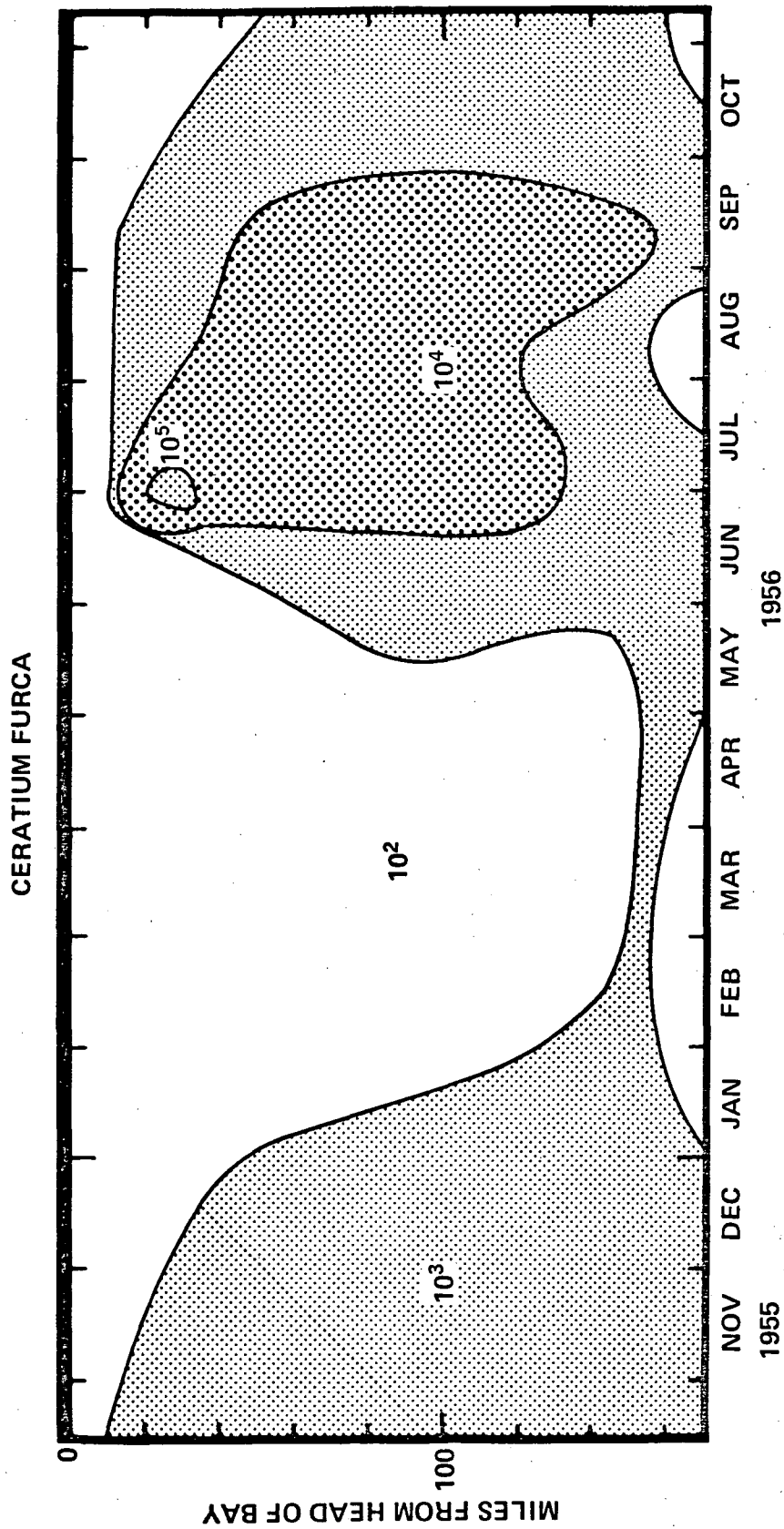
EXUVIELLA sp. PROFILE

FIGURE A-13



PERIDINIUM LEONIS PROFILE

FIGURE A-14



CERATIUM FURCA PROFILE

FIGURE A-15

vertical water turbulence. Diatoms dominate over the dinoflagella in the lower Bay while there is a rough equality of populations of these two classes in the upper Bay. The major species in the lower Bay were Skeletonema costatum and Chaetoceros affinis. It should be noted that Skeletonema has been found to be the dominant species in other east coast waters.

In March through May there are longer days, which result in warmer water. One result of this warming trend is that the diatoms were diminished as the dominant class and replaced by dinoflagella. This changeover was accompanied by a similar rise in inorganic seston and dissolved orthophosphate.

In the summer months of June-August the water reached its highest temperature and the vertical stability of the water was highest. Early in the summer the diatoms and dinoflagellates had roughly the same concentration but later in summer the diatoms were again dominant.

In the autumn there is decreasing light intensity along with lower water temperatures. Again diatoms are seen to dominate, especially in the lower Bay.

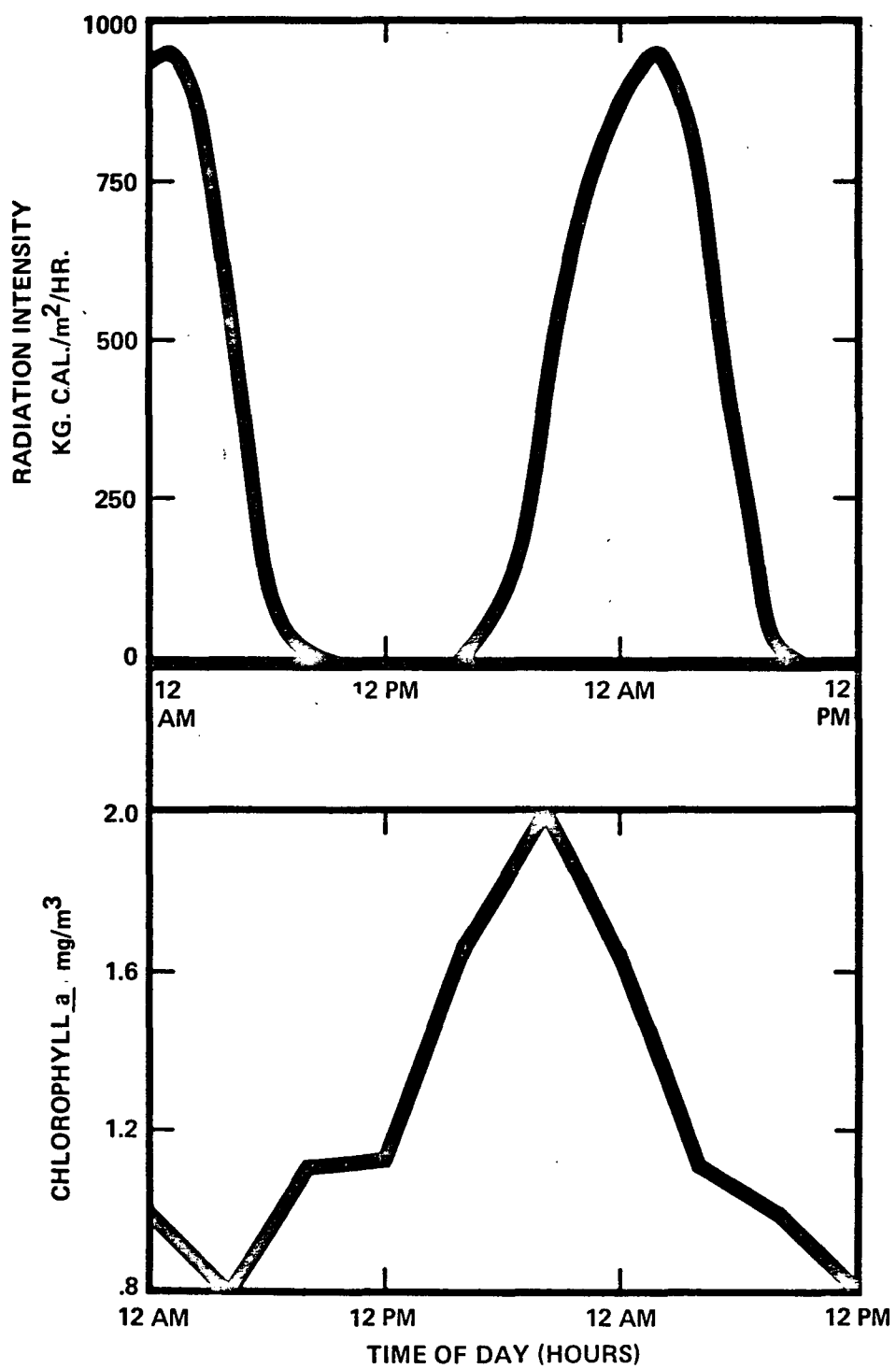
Changes in the ratio of the various species occur through selective elimination of component species and dispersion of populations through water transport. In the Chesapeake Bay, these factors are included in a yearly

cycle, which includes factors such as the effects of seasonal temperature change, nutrition availability, light intensity, etc. Other factors which may limit population growth include exhaustion of nutrients, intolerable accumulation of pollution, and disease epidemics or attacks of predatory mechanisms.

In addition to the yearly cycle, a daily cycle in chlorophyll concentration also occurs as given in Fig. A-16. This is apparently the result of bleaching of the chlorophyll during periods of intense light. During low light periods the chlorophyll reconstitutes itself. This sort of result has particular application to the remote sensing problem since it suggests that night-time operation, which is advantageous for other reasons, will require efforts to detect a substantially lower chlorophyll concentration than that which exists during daylight hours.

Further variations in time are provided by the so-called "red tides" which are fairly common in the Bay during the summer months. This red water is caused by plankton containing orange carotenes or phycobilins.

The occurrence of red water is of special interest since it represents areas of extremely high concentration of cells and further areas of special biological interest.



DIURNAL VARIATION IN CHLOROPHYLL a CONCENTRATION
IN NATURAL WATER (AFTER YENTSCH AND RYTHER (1957))

FIGURE A-16

The source of these sudden blooms is not well understood, especially in lieu of the fact that several species contribute on an individual basis to the observed blooms. Patten et al (1963) have reported aerial surveys which indicate that when "red water" is observed in one of the river systems below the Potomac, it can be found in all of them. This suggests that this is the result of simultaneous blooming under the appropriate conditions in a variety of locations. No observation of tides in the eastern side of the Bay is reported. The species which were detected include Peridinium triquetrum, Massartia rotundata, Gyrodinium aureum (there is some question about this species), Cochlodinium vinctum and Gymnodinium sp. These species are all dinoflagellates.

Strong evidence of the importance of salinity to the development of diatoms is found by inspection of Figs. A-3 and A-4. The salinity contour of 15-16‰ conforms well to the shape of the diatom distribution with both time of the year and position in the Bay except in the warmer months where the population falls substantially. Thus, it is seen that the highest concentrations occur in the lower Bay where the salinity is higher. Note however that high concentrations occur near the head of the Bay during the hottest summer months. Chaetoceros sp.

(a diatom) appears to follow the 10‰ halocline except in April and May, where the measurable concentrations are restricted to the lower 1/4 of the Bay. On the other hand, another diatom (Rhizosolenia fragilissima) appeared only in the spring months and then only in the lower Bay. Nitzschia Pungens var. Atlantica has roughly the same distribution except it is found higher in the Bay. In the case of the total dinoflagella profile there is no clear relationship between the salinity and the concentration of cells. However, for specific species there were some tendencies that appeared. For instance, there is excellent agreement between the 10‰ halocline distribution in time and space and the distribution of Prorocentrum micans. In addition, at least one dinoflagellate, Prorocentrum micans, was not observed for salinities below 10‰.

The various species have relatively complex distributions which reflect their dependence not only on temperature and salinity, but also on some of the other factors such as nutritional properties of the water, intensity and duration of sunlight and grazing by zooplankton. The salient factors which effect the concentration and distribution of algae are discussed in the following section.

3. Factors Influencing the Distribution and Growth of Algae

As seen in Section 2, the various species of plankton in the Chesapeake Bay have a complicated distribution. It is quite clear that this distribution is generated by a multitude of interacting factors, many of which are not well understood. These include air and water currents, available nutrients, flotation properties of the plankton, salinity, water temperature and turbidity. Thus, it is seen that population concentration and distribution are intimately related. For instance, if cells of a given specie are carried into regions which do not encourage their growth, the plankton will not survive in that area.

Temperature Effects:

Variations in temperature of the water have several substantial effects in the distribution and growth rate of algae.

In brackish or saline water, temperature determines the viscosity of the water and therefore plays an important part in the cell's ability to float. Sea water at 25°C offers half the resistance to sinking than do waters near 0°C. It has been further suggested that diatoms are in a most suitable growing environment when they are at just the level necessary to keep them afloat. Thus, the general observation that there is better

development of diatoms at lower levels in warm water than in cold water may be understood.

Of course, it is still true that even the most ideal conditions will result in only small concentrations of cells if the appropriate nutrients are not available. Brandt (1899) showed that the nutrient content of water depends on its temperature. Colder water limits the growth of denitrifying bacteria thus producing an abundance of nitrates.

From the work of Wolfe et al (1926) it is seen that the temperature for which diatoms are most likely to grow is between 46-55° F. This, along with the availability of nutrients combine to produce the spring and fall maxima in the diatom concentration.

Available Nutrients:

In general the nutrients in the sea are obtained from land drainage and/or are brought to the surface from deep water. Thus, the most abundant plankton growths are in coastal waters where drainage is significant and upwelling of bottom sediment is encouraged.

Discussing nutritional aspects of marine algae, Yentsch (1970) has stated that inorganic carbon, sodium, calcium, potassium, bromine, boron, magnesium, and sulfur are always available in sufficient quantities to

assure algal growth, assuming that other, more essential components are available. However, phosphorous and nitrogen are essential nutrients available in only limited amounts and their presence is critical to growth of plankton. The concentration of these elements in surface waters is greatly reduced by the growth of plankton. They are replaced by organic decomposition, and by diffusion and turbulent mixing from regions below the euphotic zone.

Patten (1963) showed that each of four main types of phosphates do exhibit seasonal variations, although their maxima did not coincide. The three nitrogen compounds that were measured in this study were nitrates, nitrites and ammonia. Although no substantial data on these nutrients was obtained in this study, the nitrates were found to be lower in the center of the Bay than in the eastern and western extremities. Nitrates appeared in maximum numbers in July through September.

Two other elements which are important to the growth of algae are calcium and magnesium. The calcium and magnesium provide the bicarbonates which are a supplemental supply of carbon dioxide for photosynthesis. In addition, the iron oxide content has a marked effect on growth rates of algae.

Phosphorous and Silicon have been found to be an important element in diatom growth (Smith, 1950) (Hentschel, 1928).

Silicon is of substantial importance in the development of diatoms since this element is contained in the cell walls

of the diatoms. Iron and Manganese have been observed to enhance cell growth in environments containing sufficient supplies of nitrogen and phosphorous. This indicates that a deficiency of Fe and Mn may limit cell growth.

Light Intensity:

One of the most important factors in the growth of any plant, including algae, is the available light. The intensity of light determines to a great extent the temperature, whose effects have been discussed. As with any plant, too much or too little light hinders growth. Thus the growth of plankton is affected by its geographical location, turbidity of the water (whether by suspended sediment or algae cells) and the intensity of the radiation at both the surface and subsurface. The depth at which cells grow is of course a result of the optimum light intensity for that specie. Light also interacts with other factors to determine the growth rate and distribution of the various plankton species.

Excessive turbulence, while it provides substantial nutrients, can also hinder growth by disturbing the plankton's ability to float and may also increase the attenuation of the light in the water to such a point that there is insufficient light available for photosynthesis.

Salinity:

The salinity is determined by tides and other natural phenomena, such as storms, etc. The apparent optimum

salinity for the growth of diatoms is 12-13‰. Since each species has its own set of optimum growing criteria when general growth is encouraged, a variety of species will alternately "bloom" to dominate the entire population.

Salinity along with the temperatures of the water also determines the distribution of the various species since each has developed unique salinity requirements as well as temperature requirements.

Other Factors:

The majority of phytoplankton are non-mobile and more dense than the surrounding water and tend to sink slowly. The cells have adapted various mechanisms to avoid sinking below the illuminated region of the water. These techniques include spines, mucilage envelopes, and oil globules. Experiments by Steele and Yentsh (1960) indicated that cells exposed to large quantities of nutrients have better flotation properties than those which are not. This could possibly result in the observed seasonal vertical motion of cells. In the spring, when nutrients are readily available, cells grow better near the surface and due to a low sinking rate remain there. In late summer, nutrients have been depleted and the sinking increases. The plants stabilize in the lower euphotic regions where nutrients are still available. It has further been noted (Steele and Yentsh, 1960) that a common Chesapeake plankton, Skeletonema costatum

has impaired flotation capability when its source of nutrition is removed.

APPENDIX B - EYE SAFETY CONSIDERATIONS

Eye safety considerations have important aspects in active laser systems since visual observation can be made of both direct and reflected signals.

Geereats (1965) states that a radiation level of 0.01 J/cm^2 at the retina corresponds to 6×10^{-8} entering the daylight adapted eye and $1.2 \times 10^{-8} \text{ J/cm}^2$ into the dark adapted eye. Kaufman (1966), using a value of 0.035 J/cm^2 at the retina, calculated a safe energy density of $2.1 \times 10^{-7} \text{ J/cm}^2$ at the cornea. The U. S. Air Force standard (1967) for pulses of 10-100 nsec is 0.125 J/cm^2 while the American Conference of Government Industrial Hygienists (1968) gives a level of 0.07 J/cm^2 .

Based on these values, Burbo, (1969) showed that the safety range R from an active laser is given by

$$R = \frac{2}{\theta} \left(\frac{J}{\pi Q} \right)^{1/2} = \frac{2}{\theta} \left(\frac{W_p T}{\pi Q} \right)^{1/2} \quad (1)$$

where W_p	=	Peak laser power
T	=	Pulse width
J	=	Energy per pulse
R	=	Range from laser
θ	=	Beam width of laser
Q	=	Threshold criteria

These calculations and the results shown in Table B-I are based on data obtained from Ruby lasers. The wavelength

of interest in this work (600nm) is considered to be close enough to the Ruby laser (694 nm) to be directly applicable to the range safety data.

Power Entering the Eye(J/cm ²)	Range for a 1MW Pulse (meters)	Range for a 200 kw pulse(meters)
2.1x10 ⁻⁷	500	245
6x10 ⁻⁸ day adapted	1090	490
1.2x10 ⁻⁸ dark adapted	2430	1080

Table B-I: Range Necessary
for Eye Safety

Since it may be advantageous to use the nitrogen laser as a source of excitation, a determination of its safety factor has been included. Using the factor that the transmission of the eye at 337 nm is approximately 1% of its transmission at 694 nm, Fantasia et al (1971) arrived at the following value of 10⁻⁵J/cm² for the maximum permissible energy density at the cornea. This energy density is equivalent to a peak power density of 1000 watts/cm² for a pulse width of 10 nsecs.

For a remote laser system the power density at the water surface is given by

$$I_L = \frac{P_L}{\Omega_L R^2} \quad (2)$$

where P_L is the laser power, Ω_L is the beam spread and R is the altitude of the aircraft above the water surface. For $P_L = 10^3 \frac{\text{watt}}{\text{cm}^2}$, $R=3 \times 10^4 \text{ cm}$ and $\Omega=4 \times 10^{-6}$ steradians, the eye safe limiting power is calculated via equation (2) as

$$P_L = 3.6 \times 10^6 \text{ watts}$$

Megawatt laser powers are state-of-the-art and as such if used indiscriminately could produce a dangerous situation to both equipment operators and observers in boats or on beaches. When the above calculations are applied to a laser emitting at a wavelength of 600 nm the eye-safe power must be reduced by roughly a factor of 100 to account for the increased transmission of the human eye. It should be noted that the tolerance level for the dark-adapted eye is much lower (by orders of magnitude) than that for the day adapted eye.

While the probability of eye damage is quite small, since the victim must look either directly at the laser or some direct reflection of the beam a potential hazard still exists. Operators of such equipment should take the necessary precautions. Although likelihood of an accident is even more remote for persons on the ground or water, the problem cannot be ignored.

APPENDIX C - LITERATURE SURVEY

Included is a list of references in the many fields which, together, form the operating system. These disciplines include quantum electronics, optics, photomultiplier tubes, fluorescence, biochemistry and electronics.

A number of fairly extensive reviews on fluorescence spectroscopy are to be found. The recent texts by White and Argauer (1972) and Udenfriend (1962) both cover the basic principles of fluorescence, as well as extensive information on the design and use of presently available commercial spectrophotofluorometers. The basic problems in experimentation in fluorescence are reviewed as well as suggesting general laboratory techniques. Udenfriend's book also includes a complete chapter on fluorescence in plants which has proved to be most helpful. Both texts also include extensive bibliographies on each of the topics considered.

White and Weissler (1972) have published several review articles for Analytical Chemistry which condense all of the information published on the topic of fluorescence (books, papers, and reports) for the period covered by the review. These articles are arranged according to the specific development or application of fluorescence and are a great aid in uncovering sources of information.

A complement to White's work is the two volume set by Passwater (1967, 1970) which presents the most complete review of modern work in fluorescence found anywhere. These volumes are cross referenced between author and topic which make them even more usable.

For a more detailed investigation into the physical principles of fluorescence and the related phenomena, the texts by Becker (1969) and Hercules (1966) should be consulted. These texts include an introduction to quantum mechanics, molecular orbital theory, calculations of transition probabilities and spin-orbit interactions. Application of these techniques is made to the various types of luminescence, including fluorescence, phosphorescence, chemiluminescence, etc.

In addition to these texts, there are many journals which regularly consider problems in fluorescence. These include Analytical Chemistry, Science, and Reviews of Scientific Instruments. Those articles of specific interest to our problem have been noted and listed in a complete bibliography at the end of the report.

There are many sources of information which deal with the biological aspects of the problem. The book edited by Lewin (1970) was found to be of great assistance since it

includes information on the botanical classification and the physiology of the various algae. In addition this book gives the biochemistry of the organisms, which includes an extensive discussion of the role of chlorophyll in the development of algae. This source includes an extensive bibliography.

An important and lengthy review article by French (1955) is specifically oriented toward the fluorescence spectroscopy of chlorophyll and the other photosynthetic pigments. This work provides data on the fluorescence of pigments in live plants as well as their properties after extraction by organic solvents.

Some journals of interest in the area of biological luminescence are Photophysiology, Annual Reviews of Plant Physiology, Nature, and Journal of Marine Research.

Another biological factor of interest to our present study is the distribution and growth of the algae of Chesapeake Bay and its environs. Appendix A has given a summary of this data, much of which has been performed by Patten (1963) and Whaley and Taylor (1968) at the Chesapeake Bay Institute of the Johns Hopkins University. However, up-to-date information on such topics as chlorophyll concentration in the Bay, available nutrients and other factors which vary from year to year can only be obtained

from correspondence or conversation with those working in the field.

Only the most recent literature on laser physics has been used. Sources of considerable interest include the journals Laser Focus, IEEE Journal of Quantum Electronics and technical information provided by the various laser manufacturers. Lengyel's (1966) book is a comprehensive and well-written review of the basic laser principles and includes chapters on applications. In addition, there are dozens of other books with varying degrees of completeness on laser systems.

A topic not so well represented in the scientific literature is that of general detection techniques and limitations. Again the Journal of Quantum Electronics presents useful material. However, Ross's (1966) book on laser receivers is most useful and complete. It includes introductory material on the general properties of laser systems, sources of noise, determination of the minimum detectable signal for a variety of photodetectors, information on the influence of the transmission media and other topics of direct application to remote detection. Of special interest to our work is the inclusion of a complete calculation of the generalized signal to noise ratio in a photodetection system. This result is used extensively in Section III.

Although there are several sources of information dealing with the problem of remote sensing of the environment, there has been little published on the applications of the laser for detection of photosynthetic pigments of plankton in the sea.

Much of the work reported on the photosynthetic components in the ocean have been obtained by using the sun as excitation source (Clark et al, 1970). The majority of the laboratory work on algae fluorescence has been performed using tungsten filament or mercury vapor lamps to excite the fluorescence. The initial use of laser light to stimulate the fluorescence of algae was reported by Hickman and Moore (1970) with additional experiments being reported by Demtröder (1971).

APPENDIX D - BIBLIOGRAPHY

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